

**(19) World Intellectual Property Organization**  
**International Bureau**



**(43) International Publication Date**  
**21 March 2002 (21.03.2002)**

**PCT**

**(10) International Publication Number**  
**WO 02/22792 A2**

**(51) International Patent Classification<sup>7</sup>: C12N 9/00**

(74) Agent: VOSSIUS & PARTNER; Siebertstrasse 4, 81675 Munich (DE).

**(21) International Application Number:** PCT/EP01/10494

(22) International Filing Date: 11 September 2001 (11.09.2001)

(25) Filing Language: English

(26) Publication Language: English

<b>(30) Priority Data:</b>		
00119849.8	12 September 2000 (12.09.2000)	EP
01113577.9	13 June 2001 (13.06.2001)	EP

**(71) Applicants and**

(72) **Inventors:** LÜSCHER, Bernhard [CH/DE]; Schwanenring 21 A, 30627 Hannover (DE). AUSTEN, Matthias [DE/US]; Wacholderweg, 24, 37079 Göttingen (DE).

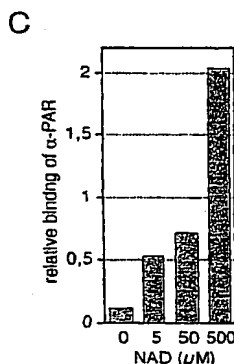
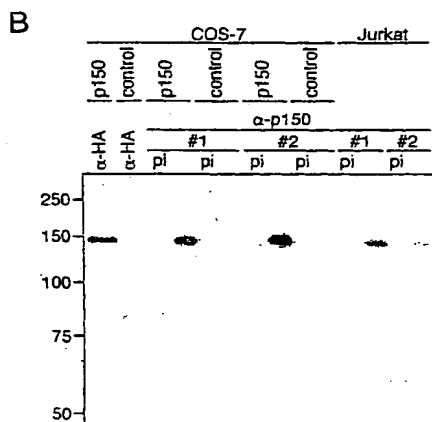
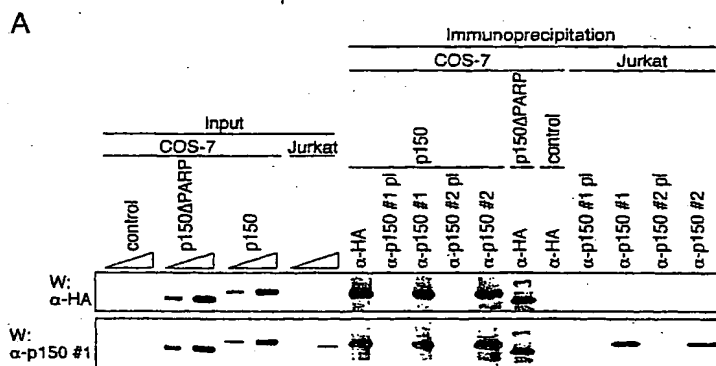
**(81) Designated States (national):** AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

**(84) Designated States (regional):** ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

*[Continued on next page]*

**(54) Title:** NUCLEIC ACID MOLECULES ENCODING A POLY(ADP-RIBOSE) POLYMERASE

**(57) Abstract:** Disclosed are nucleic acid molecules encoding a protein having poly(ADP-ribose) polymerase (PARP) activity as well as the encoded protein. Furthermore, the invention describes expression vectors, host cells, antibodies, pharmaceutical compositions and methods for treating disorders associated with aberrant regulation of cellular behaviour. Finally, screening methods are described for compounds that act as agonist or antagonist of the protein having PARP activity.



**WO 02/22792 A2**

**BEST AVAILABLE COPY**

**Published:**

- without international search report and to be republished upon receipt of that report
- with sequence listing part of description published separately in electronic form and available upon request from the International Bureau

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

## NUCLEIC ACID MOLECULES ENCODING A POLY(ADP-RIBOSE) POLYMERASE

The present invention relates to nucleic acid molecules encoding a protein having poly(ADP-ribose) polymerase (PARP) activity. The invention also relates to the encoded protein which is called p150. The invention furthermore relates to expression vectors, host cells, and an antibody for p150 as well as to pharmaceutical compositions comprising the described nucleic acid molecule, the protein, the antibody or antagonists and to methods for treating disorders associated with an aberrant regulation of cellular behaviour. The invention relates also to screening methods for compounds that act as agonist or antagonist of the protein having PARP activity.

Poly(ADP-ribosyl)ation is a post-translational modification of proteins. During this process, molecules of ADP-ribose are added successively onto acceptor proteins to form branched polymers. This modification is transient but may be very extensive *in vivo*, as polymer chains can reach more than 200 units on protein acceptors. The existence of the poly(ADP-ribose) polymer was first reported nearly 40 years ago. Since then, the importance of poly(ADP-ribose) synthesis has been established in many cellular processes (D'Amours et al., 1999)

In the recent years, several PARP enzymes, including PARP1, PARP2, tankyrase and VPARP, have been identified in mammalian cells, defining a small family of proteins (Jacobson and Jacobson, 1999). PARP1 was the first enzyme identified with PARP activity and it is by far the best studied member of this family of enzymes (D'Amours et al., 1999). PARP1 contains in addition to the PARP domain two zinc finger motifs that mediate DNA binding. PARP1 appears to function as a component of the DNA damage response mechanism in cells. This is supported by the finding that PARP1<sup>-/-</sup> mice exhibit genomic instability after treatment with certain DNA damaging agents (D'Amours et al., 1999; Jacobson and Jacobson, 1999). DNA breaks stimulate the catalytic activity of PARP1 and it is thought that the ADP-ribose polymers are a source for ATP production at the sites of DNA damage (Oei and Ziegler, 2000). In addition, PARP1 is cleaved and inactivated during apoptosis

(D'Amours et al., 1999). The recently discovered PARP2 is also activated upon DNA damage, however little is known about its biological significance (Amé et al., 1999). Together, these findings support the notion that both PARP1 and PARP2 are involved in sensing DNA damage and in ATP homeostasis important in DNA repair and in apoptosis.

Tankyrase has a catalytic domain that is related to PARP1 but otherwise no homologies to other domains of PARP family members (Smith et al., 1998). Tankyrase is associated with telomeres and it can ADP-ribosylate TRF1 which together with TRF2 is involved in stabilizing the loop structure at chromosomal ends. The addition of ADP-ribose polymers to TRF1 results in dissociation of TRF1 from telomere DNA and presumably in the disassembly of the loop structure which might be relevant for telomere maintenance. Since telomeres are important for maintaining genetic stability, tankyrase may have a function in tumorigenesis.

Vaults are large ribonucleoprotein complexes of unknown function localized primarily in the cytoplasm. Three proteins, VPARP, MVP and TEP-1, associated with these structures have been identified. VPARP contains a functional PARP domain and interacts with MVP, the major component of vaults (Kickhoefer et al., 1999). MVP is a substrate for VPARP. Interestingly, the third identified component is TEP-1 that is also part of the telomerase complex (Kickhoefer et al., 1999). VPARP is not only located in vaults but has also been found associated with the mitotic spindle. The functional relevance of this is not clear.

Of particular interest is the role of PARP enzymes in human diseases. Most of the studies published today have focused on the role of PARP1. However with the identification of additional PARPs it has become apparent that enzymes with PARP activity are most likely involved in a much broader spectrum of biological activities than previously assumed. Further studies are required to define more precisely the functions of the known PARP enzymes and also to identify additional members of the family. This has also implications for the use of drugs to modulate PARP activities.

For example, PARP proteins or antagonists thereof have been discussed to be useful in disorders related to abnormal cell death, such as cerebral ischaemia (Eliasson et al., 1997; Koedel and Pfister, 1999; Lam, 1997; Nagayama et al., 2000), diabetes (Burkart et al., 1999; Cardinal et al., 1999; Gale, 1996a; Gale, 1996b;

Pieper et al., 1999) or certain neurological or autoimmune diseases (Love et al., 1999; Cosi and Marien, 1999; Mandir et al., 1999; Tsao et al., 1999; Kroger et al., 1996).

These findings demonstrate the large scope of therapeutic potential of PARP modulators. However, previous studies have been based primarily on the original hypothesis that PARP1 is the only enzyme in mammalian cells that can poly-ADP-ribosylate proteins. With the recent identification of several additional factors with PARP activity, these new proteins have to be taken into account when findings with inhibitors are analyzed. Since it has not been studied yet in detail how the known inhibitors of PARP activity affect individual family members, it is not clear which PARP enzyme(s) is responsible for the observed effects. It is possible that these inhibitors block the activities of several PARP proteins which might have both positive and negative effects on cellular behavior. Moreover, it is at present not clear whether such inhibitors have an effect on PARP proteins that have not yet been discovered. Such obscure effects may render PARP modulating experiments highly unpredictable. Therefore, it would be desirable to get the set of PARP proteins present in mammalian cells as complete as possible in order to be able to influence more specifically cellular mechanisms related to PARP activity. More specific agonists and antagonists of PARP enzymes can then be designed to interfere with the function of specific enzymes.

Thus, the technical problem underlying the present invention is to identify other factors having PARP activity useful for improving therapeutical approaches in the context of control of cellular behavior, including proliferation, differentiation and apoptosis.

This technical problem is solved by the provision of the embodiments as characterized in the claims.

Accordingly, the present invention relates to nucleic acid molecules encoding a poly(ADP-ribose) polymerase (PARP) selected from the group consisting of

(a) nucleic acid molecules encoding a protein which comprises the amino acid sequence indicated in SEQ ID NO: 2;

- (b) nucleic acid molecules comprising the nucleotide sequence of the coding region indicated in SEQ ID NO: 1;
- (c) nucleic acid molecules encoding a protein, the amino acid sequence of which has a homology of at least 30% to the amino acid sequence indicated in SEQ ID NO: 2;
- (d) nucleic acid molecules the complementary strand of which hybridizes to a nucleic acid molecule as defined in (a) or (b); and
- (e) nucleic acid molecules, the nucleotide sequence of which deviates because of the degeneracy of the genetic code from the sequence of the nucleic acid molecules as defined in any one of (b), (c) or (d).

Consequently, the present invention relates to nucleic acid molecules encoding a protein having poly(ADP-ribose) polymerase activity, said molecules preferably encoding a protein comprising the amino acid sequence indicated in SEQ ID No: 2.

The present invention is based on the isolation of a nucleic acid molecule encoding a protein that has poly(ADP-ribose) polymerase activity and which is called p150 according to its apparent molecular weight. The nucleotide sequence is shown in SEQ ID NO: 1 and has a length of ca. 3.500 nucleotides. It encodes a protein of 1025 amino acid residues. The C-terminal 250 amino acids of the p150 protein shown in SEQ ID NO: 2 is homologous to the catalytic domain of poly(ADP-ribose) polymerases (PARP) (Figure 5). Biochemical analysis shows that this domain is functional (Example 2). The remaining portions of p150 show no significant homology to known proteins.

The p150 protein was isolated from a native c-Myc complex. Myc proteins function as transcriptional regulators and belong to a class of factors that are characterized by a basic region/helix-loop-helix/leucine zipper (bHLHZip) domain (Lüscher and Larsson, 1999). bHLHZip proteins form dimers specified by the HLHZip regions whereas the basic regions interact with DNA. Max, also a bHLHZip protein, is the obligatory heterodimerization partner of Myc proteins. Myc/Max heterodimers bind specifically to a hexameric DNA sequence with the consensus 5'-CACGTG, also referred to as Myc E box. In addition, Myc possesses a transactivation domain (TAD) that is

important for function. A number of different proteins have been identified in recent years that interact with the TAD (Dang, 1999). At least one complex that is recruited by the TAD contains histone acetyltransferase activity suggesting a function of Myc in the regulation of chromatin structure.

The functions of Myc proteins are antagonized by Mad proteins that are also bHLHZip factors and heterodimerize with Max. In particular, Mad proteins can inhibit proliferation (Foley and Eisenman, 1999; McArthur et al., 1998). This interpretation is consistent with several observations including those where the expression of mad genes is observed primarily in cells that undergo differentiation or enter a resting state and where Mad proteins repress cellular transformation (Pulverer et al., 2000). Furthermore, myeloid precursor cells of *mad1*-deficient mice show ectopic cell divisions prior to differentiation (Foley et al., 1998). Mad proteins function as transcriptional repressors by recruiting a repressor complex that includes mSin3 and histone deacetylases (Kiermaier and Eilers, 1997; Schreiber-Agus and DePinho, 1998). Thus, it is thought that Myc and Mad proteins affect cellular behavior at least in part by targeting chromatin remodeling activities to specific genes.

A number of Myc target genes have been identified that explain at least in part the role of Myc proteins in cell growth control, proliferation and tumorigenesis. These genes include ODC, CAD, cyclin D2, cdc25A, and telomerase. While in recent years several Myc interacting proteins have been identified, it is still largely unclear how Myc proteins regulate the transcription of these genes (Dang, 1999).

Some PARP proteins have also been discussed to play a role in modulation of chromatin due to their capacity to poly(ADP-ribosyl)ate core histones. For example, poly(ADP-ribosyl)ation of chromatin protein by PARP1, which is the best-studied PARP protein, causes major changes in nucleosomal architecture. Indeed, it was shown that polynucleosomes could be completely decondensed upon poly(ADP-ribosyl)ation by purified PARP1 (Poirier, Proc. Natl. Acad. Sci. U.S.A. 79 (1982), 3423-3427). According to the results of Example 2, p150 specifically poly(ADP-ribosyl)ates the core histones H2A, H2B, H3, and H4. Thus, p150 may participate in activating Myc/Max-driven transcription via its capacity to loosen up chromatin structure.

In the scope of the present invention, the term "p150" refers to a protein having PARP activity.

The term "PARP activity" refers to the enzymatic activity of adding one or more ADP-ribose monomers to a protein acceptor group, to another ADP-ribose monomer or to an ADP-ribose polymer. Preferably, said another ADP-ribose monomer or the ADP-ribose polymer are attached to a protein acceptor group.

The term "ADP-ribose polymer" refers to a molecule comprising at least two ADP-ribose monomers which are linked by a glycosidic ribose-ribose 1'→2' bond. ADP-ribose polymers comprising eight or less monomers are also referred to as "ADP-ribose oligomers". Such polymers may be branched, wherein the linkage of the branching ADP-ribose unit to the linear region is the same as between ADP-ribose monomers within the linear region. The structure of ADP-ribose polymers is, for example, described in D'Amours (1999).

The term "protein acceptor group" refers to a side chain of an amino acid residue contained in the protein to which ADP-ribose or an ADP-ribose polymer is attached by the activity of the protein of the invention. In most cases, these side chains are  $\gamma$ -carboxy groups of glutamic acid residues. ADP-ribose units may also be transferred to the side chains of other residues such as for example aspartic acid residues.

The term "adding", as well as the terms "ADP-ribosylating" or "poly(ADP-ribosyl)ating" which are used interchangeably herein, refers to a transfer reaction of ADP-ribose from nicotinamide adenine dinucleotide ( $\text{NAD}^+$ ) to a moiety mentioned above, thereby releasing nicotinamide. By this reaction, a ribose-ribose 1'→2' bond is formed if the transfer takes place to another ADP-ribose, and an ester-bond between the C1-atom of ADP-ribose and a carboxy group if the transfer takes place to a protein acceptor group.

PARP activity can, for example, be determined by assays as described in Example 2. In PARP assays, the protein can for example be tested for self-ADP-ribosylating activity (also referred to as automodification activity) by incubating the protein in a suitable buffer under suitable conditions together with  $\text{NAD}^+$  whose ADP-ribose moiety is labeled. The activity can be measured after incubation by determining the amount of labeled protein. Likewise, this assay can be used to determine the ADP-

ribosylating activity of a second protein by performing the incubation procedure as described above together with said second protein and determining the amount of said second protein which is labeled. The level of PARP activity can for instance be determined by measuring the amount of poly(ADP-ribosylation) on the substrate protein. This can for example be determined via the decrease of mobility of poly(ADP-ribosyl)ated protein during gelelectrophoresis (as shown in Figure 6D) or by detecting the nascent poly(ADP-ribose) chain directly, e.g., via the use of a poly(ADP-ribose)-specific antibody (as shown in Figure 8c).

In a preferred embodiment, the protein p150 is associated with a protein complex comprising Myc, preferably said complex comprises a Myc/Max heterodimer. Myc and Max proteins are already described in detail, above.

The term "associated" refers to non-covalent protein-protein interactions which are either direct between p150 and Myc and/or Max or indirect, i.e. mediated by at least one additional protein factor. However, preferred is a direct interaction of p150 with Myc and/or Max, preferably with Myc. The association between p150 and Myc is, for example, detectable in cell lysates, e.g. from Jurkat T cells (see Example 1). Such an association can be immunoprecipitated using, for example, Myc-specific antibodies. The protein components of such an immunoprecipitate can be analyzed using conventional SDS PAGE as shown, for example, in Figure 2. Further methods for assaying the association of two or more proteins include two-hybrid system, pull-down assays and co-immunoprecipitation as it is described in more detail further below and in Example 5. Such methods are known to the person skilled in the art and can be taken from the literature such as Lüscher-Firzlaff (Oncogene 18 (1999), 5620-5630) and references cited therein.

In a further preferred embodiment, the protein p150 has a selectivity with respect to the proteins that it is capable to ADP-ribosylate. This means that p150 ADP-ribosylates some proteins more strongly than others. For instance, it is preferred that p150 is capable of specifically ADP-ribosylating the core histones H2A, H2B, H3 and H4, as shown in Example 2. On the other hand, ADP-ribosylation of the transcription factor YY1 and bovine serum albumine is only weak.

Furthermore preferred is that the PARP activity of p150 is inhibited by 3-amino benzamide (3AB) at concentrations of at least 10 mM. Inhibition is for example detectable using a PARP assay as described above and measuring the decline of automodification activity with increasing concentrations of inhibitor (as described in Example 2).

Another preferred activity of p150 refers to its capacity to stimulate transcriptional activation which is mediated by Myc. The term "transcriptional activation" (or "transactivation" as it is also used herein) refers to a significant increase of the transcription level of a promoter upon binding of Myc to a Myc/Max recognition site present in said promoter. Preferably, p150 stimulates Myc-dependent transcriptional activation by at least two-fold. Transcriptional activation can be determined according to methods described in the literature, for instance by co-transfection assays as those described in Example 5.

A particularly preferred activity of p150 refers to its capacity to inhibit cell growth, especially cell proliferation, and/or to inhibit Myc/Ras-mediated transformation of cells. Myc/Ras-mediated transformation has been described by Cerni (1995) and Austen (1998). Preferably cell growth is inhibited due to the presence of p150, for instance by over-expressing p150 in said cells, by at least 10%, more preferably by at least 20% and most preferably by at least 40%. Likewise, it is preferred that Myc/Ras-mediated transformation is reduced by at least 20%, more preferably by at least 50%, even more preferably by at least 90% and most preferred completely due to the presence of p150 in the respective cells. Corresponding techniques to determine these activities are known to the person skilled in the art and can for instance be taken from Example 6.

In another aspect, it is preferred that p150 shows a ubiquitous expression pattern among the organs and tissues of a mammal, preferably a human, as it is apparent from Figure 9. Preferably, p150 transcription is elevated in thymus when compared to other tissues.

In another preferred embodiment the protein p150 has an apparent molecular weight in a range of between 100 and 200 kDa as determined by SDS-PAGE gel electrophoresis as described, for example, in Example 1. More preferably, this range lies between 120 and 180 kDa, even more preferably between 130 and 170 kDa, in

particular between 140 and 160 kDa, and preferably, the protein of the invention has an apparent molecular weight of 150 kDa.

The invention in particular relates to nucleic acid molecules containing the nucleotide sequence indicated under SEQ ID NO: 1 or a part thereof or a corresponding ribonucleotide sequence.

Moreover, the present invention relates to nucleic acid molecules which encode a PARP and the complementary strand of which hybridizes with one of the above-described molecules.

The present invention also relates to nucleic acid molecules which encode a protein, which has a homology, that is to say a sequence identity, of at least 30%, preferably of at least 40%, more preferably of at least 50%, even more preferably of at least 60% and particularly preferred of at least 70%, especially preferred of at least 80% and even more preferred of at least 90% to the entire amino acid sequence as indicated in SEQ ID NO: 2, the protein being a PARP.

Moreover, the present invention relates to nucleic acid molecules which encode a PARP protein and the nucleotide sequence of which has a homology, that is to say a sequence identity, of at least 40%, preferably of at least 50%, more preferably of at least 60%, even more preferably of more than 65%, in particular of at least 70%, especially preferred of at least 80%, in particular of at least 90% and even more preferred of at least 95% when compared to the coding region of the sequence shown in SEQ ID NO: 1.

The present invention also relates to nucleic acid molecules, which encode a PARP and the sequence of which deviates from the nucleotide sequences of the above-described nucleic acid molecules due to the degeneracy of the genetic code.

The invention also relates to nucleic acid molecules comprising a nucleotide sequence which is complementary to the whole or a part of one of the above-mentioned sequences.

In the context of the present invention the term "hybridization" means hybridization under conventional hybridization conditions, preferably under stringent conditions, as for instance described in Sambrook et al., Molecular Cloning, A Laboratory Manual, 2<sup>nd</sup> edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor,

NY. In an especially preferred embodiment the term "hybridization" means that hybridization occurs under the following conditions:

Hybridization buffer:	2 x SSC; 10 x Denhardt solution (Fikoll 400 + PEG + BSA; ratio 1:1:1); 0.1% SDS; 5 mM EDTA; 50 mM Na <sub>2</sub> HPO <sub>4</sub> ; 250 µg/ml of herring sperm DNA; 50 µg/ml of tRNA; or 0.25 M of sodium phosphate buffer, pH 7.2; 1 mM EDTA 7% SDS
Hybridization temperature T	= 60°C
Washing buffer:	2 x SSC; 0.1% SDS
Washing temperature T	= 60°C.

Nucleic acid molecules which hybridize with the nucleic acid molecules of the invention can, in principle, encode a PARP protein from any organism expressing such proteins or can encode modified versions thereof.

Nucleic acid molecules which hybridize with the molecules of the invention can for instance be isolated from genomic libraries or cDNA libraries of bacteria, fungi, plants or animals. Preferably, such molecules are from animal origin, particularly preferred from human origin. Alternatively, they can be prepared by genetic engineering or chemical synthesis.

Such nucleic acid molecules may be identified and isolated by using the molecules of the invention or parts of these molecules or reverse complements of these molecules, for instance by hybridization according to standard methods (see for instance Sambrook et al., 1989, Molecular Cloning. A Laboratory Manual, 2<sup>nd</sup> edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).

Nucleic acid molecules comprising the same or substantially the same nucleotide sequence as indicated in SEQ ID NO: 1 or parts thereof can, for instance, be used as hybridization probes. The fragments used as hybridization probes can also be synthetic fragments which are prepared by usual synthesis techniques, and the

sequence of which is substantially identical with that of a nucleic acid molecule according to the invention.

The molecules hybridizing with the nucleic acid molecules of the invention also comprise fragments, derivatives and allelic variants of the above-described nucleic acid molecules encoding a PARP protein. Herein, fragments are understood to mean parts of the nucleic acid molecules which are long enough to encode the described protein, preferably showing the biological activity of a PARP protein described above, e.g. being capable to add one or more ADP-ribose monomers to a protein acceptor group, another ADP-ribose or to an ADP-ribose polymer. In this context, the term derivative means that the sequences of these molecules differ from the sequences of the above-described nucleic acid molecules in one or more positions and show a high degree of homology to these sequences. In this context, homology means a sequence identity of at least 40%, in particular an identity of at least 60%, preferably of more than 65%, even more preferably of at least 70%, in particular of at least 80%, more preferably of at least 90% and particularly preferred of more than 95%. Deviations from the above-described nucleic acid molecules may have been produced, e.g., by deletion, substitution, insertion and/or recombination.

Preferably, the degree of homology is determined by comparing the respective sequence with the nucleotide sequence of the coding region of SEQ ID NO: 1. When the sequences which are compared do not have the same length, the degree of homology preferably refers to the percentage of nucleotide residues in the shorter sequence which are identical to nucleotide residues in the longer sequence. The degree of homology can be determined conventionally using known computer programs such as the DNASTAR program with the ClustalW analysis. This program can be obtained from DNASTAR, Inc., 1228 South Park Street, Madison, WI 53715 or from DNASTAR, Ltd., Abacus House, West Ealing, London W13 0AS UK (support@dnastar.com) and is accessible at the server of the EMBL outstation.

When using the Clustal analysis method to determine whether a particular sequence is, for instance, 80% identical to a reference sequence the settings are preferably as follows: Matrix: blosum 30; Open gap penalty: 10.0; Extend gap penalty: 0.05; Delay divergent: 40; Gap separation distance: 8 for comparisons of amino acid sequences. For nucleotide sequence comparisons, the Extend gap penalty is preferably set to 5.0.

Furthermore, homology means preferably that the encoded protein displays a sequence identity of at least 30%, more preferably of at least 40%, even more preferably of at least 50%, in particular of at least 60%, particularly preferred of at least 70%, especially preferred of at least 80% and even more preferred of at least 90% to the amino acid sequence depicted under SEQ ID NO: 2.

Preferably, the degree of homology of the hybridizing nucleic acid molecule is calculated over the complete length of its coding sequence. It is furthermore preferred that such a hybridizing nucleic acid molecule, and in particular the coding sequence comprised therein, has a length of at least 300 nucleotides, preferably at least 500 nucleotides, more preferably of at least 750 nucleotides, even more preferably of at least 1000 nucleotides, particularly preferred of at least 1500 nucleotides and most preferably of at least 2000 nucleotides.

Preferably, sequences hybridizing to a nucleic acid molecule according to the invention comprise a region of homology of at least 90%, preferably of at least 93%, more preferably of at least 95%, still more preferably of at least 98% and particularly preferred of at least 99% identity to an above-described nucleic acid molecule, wherein this region of homology has a length of at least 500 nucleotides, more preferably of at least 750 nucleotides, even more preferably of at least 1000 nucleotides, particularly preferred of at least 1500 nucleotides and most preferably of at least 2000 nucleotides.

Homology, moreover, means that there is a functional and/or structural equivalence between the corresponding nucleic acid molecules or proteins encoded thereby. Nucleic acid molecules which are homologous to the above-described molecules and represent derivatives of these molecules are normally variations of these molecules which represent modifications having the same biological function. They may be either naturally occurring variations, for instance sequences from other ecotypes, varieties, species, etc., or mutations, and said mutations may have formed naturally or may have been produced by deliberate mutagenesis. Furthermore, the variations may be synthetically produced sequences. The allelic variants may be naturally occurring variants or synthetically produced variants or variants produced by recombinant DNA techniques.

The proteins encoded by the different variants of the nucleic acid molecules of the invention possess certain characteristics they have in common. These include for

instance biological activity, molecular weight, immunological reactivity, conformation, etc., and physical properties, such as for instance the migration behavior in gel electrophoreses, chromatographic behavior, sedimentation coefficients, solubility, spectroscopic properties, stability, pH optimum, temperature optimum etc.

The biological activity of the PARP protein, in particular the capacity to add one or more ADP-ribose monomers to a protein acceptor group, another ADP-ribose or to an ADP-ribose polymer can be tested as described, e.g. by a PARP assay as described in Example 2.

Within the scope of the present invention are also nucleic acid molecules which comprise in addition to the coding sequence for a PARP protein, as for example the polypeptide having the amino acid sequence of SEQ ID NO: 2 or a polypeptide homologous thereto, further coding or non-coding nucleotide sequences. Thus, for instance, the nucleic acid molecule may encode the above-described polypeptide which is fused to a marker sequence, such as a peptide, which facilitates purification of the fused polypeptide. The marker sequence may for example be a hexa-histidine peptide, such as the tag provided in a pQE vector (Qiagen, Inc.) which provides for convenient purification of the fusion protein. Another suitable marker sequence may be the HA tag which corresponds to an epitope derived from influenza hemagglutinin protein (Wilson, Cell 37 (1984), 767) and which has been used in experiments described in Example 2 and Figure 7.

A further example is the coding sequence of glutathione-S-transferase (GST) that has been used in experiments described in Example 2. Apart from providing a purification tag, the fusion to GST enhances protein stability, for instance, in bacterial expression systems.

The nucleic acid molecules of the invention can be DNA molecules, in particular genomic DNA or cDNA. Moreover, the nucleic acid molecules of the invention may be RNA molecules. The nucleic acid molecules of the invention can be obtained for instance from natural sources or may be produced synthetically or by recombinant techniques, such as PCR.

The nucleic acid molecules of the invention now allow host cells to be prepared which produce recombinant proteins having PARP activity of high purity and/or in sufficient quantities, as well as genetically engineered cells possessing an increased or reduced activity of these proteins.

The invention also relates to oligonucleotides specifically hybridizing to a nucleic acid molecule of the invention. Such oligonucleotides have a length of preferably at least 10, in particular at least 15, and particularly preferably of at least 50 nucleotides. They are characterized in that they specifically hybridize to the nucleic acid molecules of the invention, that is to say that they do not or only to a very minor extent hybridize to nucleic acid sequences encoding other PARP proteins. The oligonucleotides of the invention can be used for instance as primers for amplification techniques such as the PCR reaction or as a hybridization probe to isolate related genes.

Moreover, the invention relates to vectors, in particular plasmids, cosmids, viruses, bacteriophages and other vectors commonly used in genetic engineering, which contain the above-described nucleic acid molecules of the invention. In a preferred embodiment of the invention, the vectors of the invention are suitable for the transformation of fungal cells, cells of microorganisms or animal cells, in particular mammalian cells. Preferably, such vectors are suitable for the transformation of human cells. In a particularly preferred embodiment such vectors are suitable for gene therapy purposes.

In another preferred embodiment, the nucleic acid molecules contained in the vectors are connected to regulatory elements ensuring transcription and synthesis of a translatable RNA in prokaryotic or eukaryotic cells.

The expression of the nucleic acid molecules of the invention in prokaryotic or eukaryotic cells, for instance in *Escherichia coli*, is interesting because it permits a more precise characterization of the biological activities of the proteins encoded by these molecules. Moreover, it is possible to express these proteins in such prokaryotic or eukaryotic cells which are free from interfering proteins. In addition, it is possible to insert different mutations into the nucleic acid molecules by methods

usual in molecular biology (see for instance Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, 2<sup>nd</sup> edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY), leading to the synthesis of proteins possibly having modified biological properties. In this regard it is on the one hand possible to produce deletion mutants in which nucleic acid molecules are produced by progressive deletions from the 5' or 3' end of the coding DNA sequence, and said nucleic acid molecules lead to the synthesis of correspondingly shortened proteins.

On the other hand, the introduction of point mutations is also conceivable at positions at which a modification of the amino acid sequence for instance influences the biological activity or the regulation of the protein.

Moreover, mutants possessing a modified substrate or product specificity can be prepared. Furthermore, it is possible to prepare mutants having a modified activity-temperature-profile.

Furthermore, in the case of expression in human cells, the introduction of mutations into the nucleic acid molecules of the invention allows the gene expression rate and/or the activity of the proteins encoded by the nucleic acid molecules of the invention to be reduced or increased.

For genetic engineering in prokaryotic cells, the nucleic acid molecules of the invention or parts of these molecules can be introduced into plasmids which permit mutagenesis or sequence modification by recombination of DNA sequences. Standard methods (see Sambrook et al., 1989, Molecular Cloning: A laboratory manual, 2<sup>nd</sup> edition, Cold Spring Harbor Laboratory Press, NY, USA) allow base exchanges to be performed or natural or synthetic sequences to be added. DNA fragments can be connected to each other by applying adapters and linkers to the fragments. Moreover, engineering measures which provide suitable restriction sites or remove surplus DNA or restriction sites can be used. In those cases, in which insertions, deletions or substitutions are possible, in vitro mutagenesis, "primer repair", restriction or ligation can be used. In general, a sequence analysis, restriction analysis and other methods of biochemistry and molecular biology are carried out as analysis methods.

Another embodiment of the invention relates to host cells, in particular prokaryotic or eukaryotic cells transformed and/or genetically modified with an above-described

nucleic acid molecule of the invention or with a vector of the invention, and to cells derived from such transformed cells and containing a nucleic acid molecule or vector of the invention. In a preferred embodiment the host cell is genetically modified in such a way that it contains a nucleic acid molecule stably integrated into the genome. More preferably the nucleic acid molecule can be expressed so as to lead to the production of a protein having the biological activity of a PARP protein.

An overview of different expression systems is for instance contained in Methods in Enzymology 153 (1987), 385-516, in Bitter et al. (Methods in Enzymology 153 (1987), 516-544) and in Sawers et al. (Applied Microbiology and Biotechnology 46 (1996), 1-9), Billman-Jacobe (Current Opinion in Biotechnology 7 (1996), 500-4), Hockney (Trends in Biotechnology 12 (1994), 456-463), Griffiths et al., (Methods in Molecular Biology 75 (1997), 427-440). An overview of yeast expression systems is for instance given by Hensing et al. (Antonie van Leeuwenhoek 67 (1995), 261-279), Bussineau et al. (Developments in Biological Standardization 83 (1994), 13-19), Gellissen et al. (Antonie van Leeuwenhoek 62 (1992), 79-93), Fleer (Current Opinion in Biotechnology 3 (1992), 486-496), Vedvick (Current Opinion in Biotechnology 2 (1991), 742-745) and Buckholz (Bio/Technology 9 (1991), 1067-1072).

Expression vectors have been widely described in the literature. As a rule, they contain not only a selection marker gene and a replication-origin ensuring replication in the host selected, but also a bacterial or viral promoter, and in most cases a termination signal for transcription. Between the promoter and the termination signal there is in general at least one restriction site or a polylinker which enables the insertion of a coding DNA sequence. The DNA sequence naturally controlling the transcription of the corresponding gene can be used as the promoter sequence, if it is active in the selected host organism. However, this sequence can also be exchanged for other promoter sequences. It is possible to use promoters ensuring constitutive expression of the gene and inducible promoters which permit a deliberate control of the expression of the gene. Bacterial and viral promoter sequences possessing these properties are described in detail in the literature. Regulatory sequences for the expression in microorganisms (for instance *E. coli*, *S. cerevisiae*) are sufficiently described in the literature. Promoters permitting a particularly high expression of a downstream sequence are for instance the T7 promoter (Studier et al., Methods in Enzymology 185 (1990), 60-89), lacUV5, trp, trp-

lacUV5 (DeBoer et al., in Rodriguez and Chamberlin (Eds), Promoters, Structure and Function; Praeger, New York, (1982), 462-481; DeBoer et al., Proc. Natl. Acad. Sci. USA (1983), 21-25), *l*p1, *rac* (Boros et al., Gene 42 (1986), 97-100). Inducible promoters are preferably used for the synthesis of proteins. These promoters often lead to higher protein yields than do constitutive promoters. In order to obtain an optimum amount of protein, a two-stage process is often used. First, the host cells are cultured under optimum conditions up to a relatively high cell density. In the second step, transcription is induced depending on the type of promoter used. In this regard, a *tac* promoter is particularly suitable which can be induced by lactose or IPTG (=isopropyl- $\beta$ -D-thiogalactopyranoside) (deBoer et al., Proc. Natl. Acad. Sci. USA 80 (1983), 21-25). Termination signals for transcription are also described in the literature.

The transformation of the host cell with a nucleic acid molecule or vector according to the invention can be carried out by standard methods, as for instance described in Sambrook et al., (Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> edition (1989) Cold Spring Harbor Press, New York; Methods in Yeast Genetics, A Laboratory Course Manual, Cold Spring Harbor Laboratory Press, 1990). The host cell is cultured in nutrient media meeting the requirements of the particular host cell used, in particular in respect of the pH value, temperature, salt concentration, aeration, antibiotics, vitamins, trace elements etc. The protein according to the present invention can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

Moreover, the invention relates to a protein and biologically active fragments thereof, which is encoded by a nucleic acid molecule according to the invention and to methods for its preparation, wherein a host cell according to the invention is cultured under conditions permitting the synthesis of the protein, and the protein is subsequently isolated from the cultured cells and/or the culture medium. The present

invention also relates to the protein obtainable by such a method. The protein of the present invention may, e.g., be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the protein of the present invention may be glycosylated or may be non-glycosylated. The protein of the invention may also include an initial methionine amino acid residue. The protein according to the invention may be further modified to contain additional chemical moieties not normally part of the protein. Those derivatized moieties may, e.g., improve the stability, solubility, the biological half life or absorption of the protein. The moieties may also reduce or eliminate any undesirable side effects of the protein and the like. An overview for these moieties can be found, e.g., in Remington's Pharmaceutical Sciences (18<sup>th</sup> ed., Mack Publishing Co., Easton, PA (1990)). Polyethylene glycol (PEG) is an example for such a chemical moiety which has been used for the preparation of therapeutic proteins. The attachment of PEG to proteins has been shown to protect them against proteolysis (Sada et al., J. Fermentation Bioengineering 71 (1991), 137-139). Various methods are available for the attachment of certain PEG moieties to proteins (for review see: Abuchowski et al., in "Enzymes as Drugs"; Holcerberg and Roberts, eds. (1981), 367-383). Generally, PEG molecules are connected to the protein via a reactive group found on the protein. Amino groups, e.g. on lysines or the amino terminus of the protein are convenient for this attachment among others.

Furthermore, the present invention also relates to an antibody specifically recognizing a protein according to the invention. The antibody can be monoclonal or polyclonal and can be prepared according to methods well known in the art. The term "antibody" also comprises fragments of an antibody which still retain the binding specificity.

The protein according to the invention, its fragments or other derivatives thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. The present invention in particular also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab

expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

Antibodies directed against a protein according to the present invention can be obtained, e.g., by direct injection of the protein into an animal or by administering the protein to an animal, preferably a non-human animal. The antibody so obtained will then bind the protein itself. In this manner, even a sequence encoding only a fragment of the protein can be used to generate antibodies binding the whole native protein. Such antibodies can then, e.g., be used to isolate the protein from tissue expressing that polypeptide or to detect it in a probe. For the preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples for such techniques include the hybridoma technique (Köhler and Milstein, *Nature* 256 (1975), 495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., *Immunology Today* 4 (1983), 72) and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. (1985), 77-96). Techniques describing the production of single chain antibodies (e.g., U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to immunogenic proteins according to the present invention. Furthermore, transgenic mice may be used to express humanized antibodies directed against immunogenic proteins of the present invention.

The present invention also relates to a pharmaceutical composition comprising a protein according to the invention, a nucleic acid molecule according to the invention or an agonist of said protein which is suitable to express such a protein in target cells, and optionally a pharmaceutically acceptable carrier or excipient.

Moreover, the present invention also relates to the use of a protein according to the invention, a nucleic acid molecule according to the invention or an agonist of said protein which is suitable to express such a protein in target cells for the preparation of a pharmaceutical composition for the prevention and/or treatment of tumors.

With the association of PARP enzymes, as is in particular known from PARP1 and PARP2, with DNA damage and repair and cell death, modulators of PARP activity may be relevant in tumor therapy. If such a pathological condition is associated with

an aberrantly reduced PARP activity, administration of pharmaceutical compositions comprising a protein or a nucleic acid molecule of the invention to a patient may ameliorate this condition. Furthermore, the treatment of malignant cells with combinations of drugs may become more effective and/or more selective when such pharmaceutical compositions modulating PARP activity are included.

As already mentioned, a preferred embodiment of the present invention refers to the association of the protein of the invention with a protein complex comprising Myc, for example c-Myc. The proteins belonging to the Myc-related network are involved in the regulation of cellular behaviour, including proliferation, differentiation and apoptosis which is fundamental to development and homeostasis of organisms (Dang, 1999; Henriksson and Lüscher, 1996). In particular, Myc proteins which are expressed preferentially in cycling cells promote cell growth and inhibit terminal differentiation. The expression of *myc* genes is frequently deregulated in tumors due to genetic alterations and Myc proteins are thought to contribute to both tumor development and progression (Nesbit et al., 1999). Several lines of experimental evidence support this interpretation. Myc together with an activated form of Ras is able to transform primary rodent cells. In addition, retroviruses carrying a *myc* gene are highly oncogenic in chicken and the expression of Myc in different tissues of transgenic mice is sufficient to significantly increase tumor incidence in these animals. Together these findings strongly support the concept that Myc has oncogenic potential, most likely due to its capacity to affect growth and differentiation of cells.

Although the biochemical and functional aspects of the interaction of p150 with c-Myc have so far not been analyzed in detail, it is pointed out that PARP activity has been suggested to be relevant in the regulation of gene expression (D'Amours et al., 1999). The potential of p150 to modify core histones suggests that this protein may be involved in regulating chromatin structure and through this affect the expression of genes. An additional aspect is that Myc proteins have been shown to induce genetic instability (Felsner and Bishop, 1999). Since PARP1, PARP2 and tankyrase have been implicated in DNA repair and genetic stability, modification of proteins by poly-ADP-ribosylation may be of general importance for maintaining genetic stability and, thus, p150 may be relevant for this function of Myc proteins.

If it should turn out that p150 modulates under certain circumstances Myc-mediated gene expression, administering a protein of the invention or a nucleic acid molecule encoding and capable of expressing said protein to cells or tissues, in which Myc-mediated gene expression is abnormal, may ameliorate the pathological consequences of the abnormal gene expression. Thus, pathological conditions which are related to an altered, preferably a reduced ratio of Myc to p150 in cells may be treated or prevented using pharmaceutical compositions comprising the protein or the nucleic acid molecule of the invention. Thereby, malignant transformation of cells may be prevented or uncontrolled proliferation of cells leading to growth of tumors or other neoplasies may be inhibited.

However, as it has been shown experimentally in Example 6, overexpression of p150 leads to an inhibition of cell growth and abolishes Myc/Ras-induced transformation of previously non-tumor cells (e.g. primary rat embryo fibroblasts). These functions might be independent of the function of p150 to stimulate Myc-dependent transcriptional activation (Example 5). Accordingly, p150 or nucleic acid molecules encoding it and capable of being expressed in target cells have a particularly strong potential in preventing and/or treating neoplasia such as tumors, especially when neoplasia is Myc/Ras-induced. Such a potential may likewise apply to agonists of p150 which, for example, increase endogenous p150 activity in cells.

The present invention therefore also relates to a method for treating or preventing tumors in a patient comprising administering a suitable amount of the protein of the invention, a nucleic acid molecule encoding it and capable of being expressed in appropriate target cells of said patient, or an agonist of said protein to said patient. The term "agonist" refers in connection with the present invention to a compound that increases p150 activity and may for example include compounds that induce an increase of the endogenous p150 in cells to which they are applied. Likewise, such an agonist compound may have the property to inhibit or at least to reduce the interaction of p150 with Myc. Suitable agonist compounds may be provided using a screening method as described infra. Preferably the compounds mentioned above, i.e. protein, nucleic acid molecule or agonist, are formulated in a pharmaceutical composition as it is described herein.

Furthermore, the provision of the protein of the invention furthermore allows to develop more specific prophylactic or therapeutic uses directed to PARP activity.

Previous studies have been based on the original hypothesis that PARP1 is the only poly(ADP-ribosyl)ating activity in mammalian cells. Therefore, therapeutic methods that modify PARP activity which are for example directed to PARP1 may also affect other PARP enzymes. The description of additional PARP enzymes facilitates the development of therapeutic approaches that specifically address one PARP enzyme. In this context, the disclosure of the protein of the invention contributes to the development of more specific and therefore more effective means and methods to modify PARP activity with benefits, for example, for anti-tumor prevention and/or treatment. Such means and methods may for example include the screening for compounds that act as agonists or antagonists of proteins having PARP activity, preferably of the protein of the invention.

Moreover, the knowledge of as many as possible PARP proteins makes it possible to prevent or alleviate negative side effects of compounds that affect the activity of more than one PARP protein. If, for example, an inhibitor of PARP1 is beneficial but, as a negative side effect, also inhibits p150, the addition of an agonist capable of specifically activating p150 could compensate for the negative side effect of the inhibitor. Thus, the protein of the invention is suited to enhance prevention and/or treatment, for example, of cancer, also regarding the improvement of already known approaches.

The pharmaceutical compositions according to the invention may be suitable to be administered orally, rectally, parenterally, intracisternally, intradermally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, creams, drops or transdermal patch), buccally, or as an oral or nasal spray. By "pharmaceutically acceptable carrier or excipient" is meant a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion. It is understood that, when administered to a human patient, the total daily usage of the pharmaceutical compositions of the present invention will be decided by the attending physician within the scope of sound medical judgement. The specific therapeutically effective dose level for any particular patient will naturally depend upon a variety of factors including the age,

body weight, general health, sex and diet of the patient, the time of administration, route of administration, and rate of excretion of the composition; the duration of the treatment, drugs (such as a chemotherapeutic agent) used in combination or coincidental with the specific composition and the like. Suitable formulations can be found, e.g., in Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA.

The protein according to the present invention may also be employed by expression of such a protein in vivo, which is also referred to as "gene therapy".

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) encoding a protein ex vivo, with the engineered cells then being provided to a patient to be treated with the protein. Such methods are well known in the art. For example, cells may be engineered by procedures known in the art by use of a retroviral particle containing RNA encoding a protein according to the present invention.

The present invention also relates to an antagonist of the protein according to the invention. Examples of antagonists are an antibody according to the invention or an antisense construct directed against a transcript of a nucleic acid molecule according to the invention or a nucleotide sequence encoding such an antisense construct.

The antisense approach is an approach well known in the art and it can be used to control gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of a nucleotide sequence which codes for the protein of the present invention is used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. The antisense RNA oligonucleotide hybridizes to the corresponding RNA in vivo and blocks translation of the RNA molecule. The antisense oligonucleotides described above can be delivered directly into cells or corresponding nucleotide sequences may be delivered into the cells such that the antisense RNA or DNA may be expressed in vivo.

Another example for an antagonist of the protein according to the invention are fragments of the p150 protein which are still capable of associating with a Myc-comprising protein complex and which do no longer lead to the normal biological response of said protein complex. Such fragments of the p150 protein can readily be determined by the person skilled in the art, e.g., by preparing corresponding deletion

mutants and testing these mutants in an assay for PARP activity as described in Example 2 and in a second assay for their ability to form an association with Myc. The second assay may be carried out using yeast two hybrid systems; pull-down assays and/or coimmunoprecipitations. All the three methods are described in Lüscher-Firzlaff (Oncogene 18 (1999), 5620-5630) and references cited therein. For a yeast two hybrid system, different parts of either Myc or p150 can be used as bait or prey; the interaction is then determined by measuring the activity of a reporter gene. Pull-down assays can be performed by expressing either Myc or p150 and/or fragments thereof as GST-fusion proteins (see also Example 5). These are then bound to glutathione-agarose beads. The interaction partners (full length proteins or mutants) to be tested are obtained by in vitro transcription/translation whereby binding to the immobilized fusion proteins can then be determined. Coimmunoprecipitations can be carried out by coexpressing either the protein or fragments thereof as tagged versions (e.g. HA, FLAG) in COS7 cells (see also Example 5). Protein complexes are extracted under low stringent conditions and immunoprecipitated using antibodies specific for one component of the complex, for instance to the tag. The association of other components can then be determined by Western blot analysis.

If the p150 binding partners are different from Myc, such as other protein factors or specific nucleic acid molecules, the above described activity and/or binding assays may be used correspondingly for preparing antagonistic fragments of p150.

Likewise, an antagonist of p150 can also be a fragment of the binding partner to which p150 is normally bound, as for example Myc. Such fragments are capable of specifically binding to p150 but do not facilitate its activity which normally takes place upon binding. Such fragments can be prepared according to the above-described methods.

Further examples for antagonists are compounds that inhibit PARP activity, such as 3AB as described in Example 2.

The present invention also relates to a pharmaceutical composition comprising an antagonist of the protein according to the invention and optionally a pharmaceutically acceptable carrier or excipient.

The present invention furthermore relates to the use of an antagonist of the protein according to the invention for the preparation of a pharmaceutical composition for the treatment of cerebral ischaemia, diabetes, Alzheimer's disease, Parkinson's disease, systemic lupus erythematosus (SLE) or arthritis or for the prevention and/or treatment of tumors.

The application of inhibitors or antagonists to PARP activity has already gained considerable attention. It is conceivable that at least some of these potential applications may also be of relevance with regard to the protein of the invention. For example, it has been reported that the activation of PARP1 leads to a rapid depletion of NAD<sup>+</sup> and subsequently of ATP in cells (D'Amours et al., 1999), which can trigger cell death. Interestingly, the inhibition of PARP1 or the inactivation of this enzyme in PARP1<sup>-/-</sup> mice results in a decreased loss of cells upon cerebral ischaemia and other forms of stress (Eliasson et al., 1997; Koedel and Pfister, 1999; Lam, 1997; Nagayama et al., 2000). Furthermore, PARP enzymes appear also to be relevant in diabetes. The experimental destruction of the insulin producing cells in the pancreas is reduced by PARP inhibitors as well as in PARP1<sup>-/-</sup> mice (Burkart et al., 1999; Cardinal et al., 1999; Gale, 1996a; Gale, 1996b; Pieper et al., 1999). Of interest is also the finding that neurons of patients with Alzheimer's disease have increased PARP activity and it has been argued that PARP antagonists might be beneficial for such patients (Love et al., 1999). Furthermore, it has been suggested that inhibition of PARP activity could be advantageous for individuals with Parkinson's disease (Cosi and Marien, 1999; Mandir et al., 1999). Interestingly, the autoimmune disease SLE (systemic lupus erythematosus) shows genetic linkage to chromosome 1q41-42. Since the gene encoding PARP1 is localized in this chromosomal region it has been hypothesized that PARP1 might be the SLE susceptibility gene (Tsao et al., 1999). In this respect, it is relevant that in a mouse model for arthritis, another autoimmune disease, PARP antagonists are inhibitory (Kroger et al., 1996).

Another field for applying antagonists may be seen in preventing and/or treating tumors. This is in line with the observations regarding the association of p150 with Myc proteins which may lead to promotion and/or growth of tumors, as discussed above. Thus, if it should turn out that p150 modulates Myc-mediated gene expression, administering an antagonist of p150 or, if the antagonist is producible by

transcription and/or translation, a nucleic acid molecule encoding the antagonist to cells or tissues affected with abnormal Myc-mediated gene expression may be of therapeutical benefits. Preferably, this antagonist or nucleic acid molecule encoding an antagonist is administered if abnormal Myc function is associated with an aberrantly increased level of p150 or p150 activity.

The present invention furthermore provides a method for screening compounds to identify those which act as agonists or antagonists of the protein according to the invention. Such a method preferably comprises the steps of

- (a) measuring the ADP-ribosylating activity of a protein of the invention in the presence and in the absence of the compound to be tested; and
- (b) determining that the activity measured in step (a) is
  - (i) higher in the presence of the compound than in the absence of the compound, thereby identifying a compound acting as agonist; or
  - (ii) lower in the presence of the compound than in the absence of the compound, thereby identifying a compound acting as antagonist.

Preferably, the protein of the invention used in this method is recombinantly produced via expression, for instance, in bacteria or insect cells. Advantageously, the protein is attached to a solid support using, e.g. a tag (such as for example HA or FLAG) or antibodies. The ADP-ribosylating activity is preferably measured with the protein itself as substrate (automodification). Suitable reaction conditions and buffers are for instance given in the examples. Preferably, ADP-ribosylation activity is measured using a monoclonal antibody specific for poly(ADP-ribose).

Furthermore, the present invention relates to a method for preparing a pharmaceutical composition comprising the steps of identifying an agonist or an antagonist of the protein by one of the methods described above and formulating the identified agonist or antagonist in a pharmaceutical composition. For the formulation of the pharmaceutical composition the same applies as described above for the pharmaceutical compositions according to the invention.

Moreover, the present invention relates to a diagnostic kit or composition comprising the protein, the oligonucleotide, the nucleic acid molecule or the antibody according to the invention.

The oligonucleotide or the nucleic acid molecule according to the invention may, e.g., be used for determining whether an individual carries a mutation in the gene encoding p150, thereby allowing to determine whether the individual suffers from a disease or has the susceptibility for a disease related to the presence of mutations in the p150 gene.

A variety of techniques is available to detect mutations at the DNA level. For example, nucleic acids for diagnosis may be obtained from a patient's cells, such as from blood, urine, saliva, tissue biopsy and autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR prior to analysis. RNA or cDNA may also be used for the same purpose. As an example, PCR primers complementary to the nucleic acid molecule encoding the p150 protein can be used to identify and analyze mutations of p150. Deletions and insertions can, e.g., be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled p150 RNA or alternatively, radiolabeled p150 antisense DNA sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures.

Genetic testing based on DNA sequence differences may be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis. DNA fragments of different sequences may be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperature.

The protein and/or the antibody according to the present invention can, e.g., be used in a diagnostic assay for detecting altered levels of a protein according to the invention in various tissues. An over- or under-expression of the protein compared to normal control tissue samples may detect the presence of a disease or susceptibility to a disease. Suitable assays are well known in the art and include

radioimmunoassays, competitive-binding assays, Western Blot analysis, ELISA assays and "sandwich" assay.

These and other embodiments are disclosed and obvious to a skilled person and embraced by the description and the examples of the present invention. Additional literature regarding one of the above-mentioned methods, means and applications, which can be used within the meaning of the present invention, can be obtained from the state of the art, for instance from public libraries for instance by the use of electronic means. This purpose can be served inter alia by public databases, such as the "medline", which are accessible via internet, for instance under the address <http://www.ncbi.nlm.nih.gov/PubMed/medline.html>. Other databases and addresses are known to a skilled person and can be obtained from the internet, for instance under the address <http://www.lycos.com>. An overview of sources and information regarding patents and patent applications in biotechnology is contained in Berks, TIBTECH 12 (1994), 352-364.

All of the above cited disclosures of patents, publications and database entries are specifically incorporated herein by reference in their entirety to the same extent as if each such individual patent, publication or entry were specifically and individually indicated to be incorporated by reference.

**Figure 1** c-Myc/Max are part of high molecular weight complexes.

Exponentially growing Jurkat T cells were lysed in F-buffer under conditions which preserve DNA binding competent c-Myc/Max heterodimers. These lysates were chromatographed over a Sepharose CL-6B sizing column. Aliquots of the fractions were analyzed for c-Myc levels by Western blot analysis using a polyclonal serum recognizing c-Myc (SC N262). Both p67 and p64, corresponding to the two major translational products, are visible. In a parallel experiment the expression of Max p21 and Max p22 was measured using the Max-specific serum 85. The column was calibrated using the following size markers: thyroglobulin 670 kDa, ferritin 440 kDa, catalase 230 kDa, aldolase 156 kDa, and bovine serum albumin 70 kDa. The peak fractions containing these markers are indicated.

**Figure 2** c-Myc-associated proteins.

F-buffer lysates of  $\sim 10^9$  Jurkat cells were chromatographed over affinity columns containing either 50  $\mu$ g of mAb 6A10 (specific for human c-Myc) or mAb 5C9 (specific for human Mad1) as indicated in the figure. The eluted proteins were separated on SDS-PAGE and analyzed by silver staining. c-Myc and the three associated proteins are indicated by arrowheads.

**Figure 3** p150 cDNA and protein sequence.

A cDNA was assembled from different EST clones (see Material and Methods for details), sequenced and an open reading frame defined, coding for a protein of 1025 amino acids. The protein sequence derived from the assembled cDNA is consistent with the peptide sequences derived from the purified p150 protein. The corresponding peptide sequences are underlined.

**Figure 4** p150 mRNA and protein.

A. The mRNA size of p150 was determined by Northern blot analysis. A single mRNA species of about 3.7 kb was detected.

B. The assembled cDNA was transcribed and translated in the presence of  $^{35}$ S-methionine in vitro and the resulting protein analyzed by SDS-PAGE. A single protein species of 150 kDa was detected. As controls luciferase and Ash2 were analyzed in parallel.

**Figure 5** The C-terminus of p150 shows homology to the catalytic domains of PARP enzymes.

Amino acids 881-1012 of p150 are compared to the catalytic domains of several PARP enzymes, including tankyrase (aa 1178-1316), p193 VPARP (aa 434-572), PARP1 (aa 856-1013), and PARP2 (aa 409-569) (Amé et al., 1999; Cherney et al., 1987; Kickhoefer et al., 1999; Smith et al., 1998) using ClustalW as described above. Amino acids that are

identical in at least three of the five sequences are dark-shaded. Amino acids showing a similarity in at least three sequences are light-shaded. The sequence identity between the PARP domain of p150 and its homologues is 20% for tankyrase, 16% for p193 VPARP, and 13% for PARP1 and PARP2, respectively. The corresponding cDNA sequence of p150 has an identity of 39% compared to tankyrase. Structural elements deduced from the known crystal structure of PARP1 are indicated (Ruf et al., 1996). L refers to a potential  $\alpha$ -helical segment whereas c to n refer to  $\beta$ -sheets.

**Figure 6** The bacterially expressed C-terminal domain of p150 has ADP-ribose polymerase activity.

A. Amino acids 519-1025 of p150 were expressed as a GST fusion protein (GST-p150C) in *E. coli* and purified using glutathione agarose. A protein of about 100 kDa was identified that corresponds to the full length fusion protein. Several break down products are visible (indicated by asterisks). A coomassie blue stained gel is shown.

B. Bacterially expressed GST-p150C or GST alone (control) was incubated with  $^{32}\text{P}$ -NAD $^{+}$ . The labeled proteins were separated on SDS-PAGE gels and the proteins visualized by autoradiography. Note that GST-p150C is labeled but none of the breakdown products.

C. Core histones (3  $\mu\text{g}$ ) were incubated with GST-p150C as indicated. All four core histones were ADP-ribosylated.

D. GST-p150C was incubated in the presence of  $^{32}\text{P}$ -NAD $^{+}$  and increasing amounts of unlabeled NAD $^{+}$  as indicated. A decreased mobility in the range of 10 to 15 kDa is observed.

E. GST-p150C was incubated with  $^{32}\text{P}$ -NAD $^{+}$  in the presence of the indicated amounts of 3-amino benzamide (3AB).

**Figure 7** Full length p150 possesses PARP activity.

The full length p150 was expressed transiently in COS7 cells as an HA-tagged version of p150.

A. Western blot analysis of whole cell extracts with an mAb recognizing the HA-tag revealed a single band of 150 kDa. No specific signal was seen in control cell extracts.

B. HA-tagged p150 was immunoprecipitated with HA-specific mAbs and then incubated with  $^{32}\text{P}$ -NAD $^{+}$ . For control, an immunoprecipitate from control cell lysates was used. A specific band of 150 kDa could be detected in the p150 immunoprecipitate but none in the control.

**Figure 8** Characterization of two antisera that recognize p150 and immunoprecipitate PARP activity.

A. COS-7 cells were transiently transfected with expression plasmids encoding HA-tagged p150, HA-tagged p150 with a deleted PARP domain containing amino acid residues 1 to 922 of SEQ ID NO:2 which does not show PARP activity (p150 $\Delta$ PARP), or with empty vector (control). From these transfected cells as well as from Jurkat T cells, F buffer lysates were produced as described before (Sommer, 1998). Aliquots of these lysates were analyzed for the presence of transiently expressed p150 and p150 $\Delta$ PARP or for endogenous p150 (Input).

p150 proteins were immunoprecipitated either from COS-7 lysates or from Jurkat cell lysates using an HA-specific monoclonal antibody ( $\alpha$ -HA) or p150 specific rabbit sera #1 or #2 (Immunoprecipitation). One set of samples was probed with  $\alpha$ -HA, the other set with  $\alpha$ -p150 #1, as indicated. W: Western blot; pi: preimmune serum.

B. Transiently expressed p150 from COS-7 cell lysates, from control COS-7 cell lysates and endogenous p150 from Jurkat T cells was immunoprecipitated using the antibodies mentioned under A. The precipitates were tested for PARP activity (automodification). An autoradiogram is shown.

C. To obtain further evidence for the formation of poly(ADP-ribose) (PAR), GST-p150C was incubated with  $^{32}\text{P}$ -NAD $^{+}$  and an increasing amount of unlabeled NAD $^{+}$ . Each sample was then divided into three. One was immunoprecipitated with serum #1 (1), one with preimmune

serum #1 (2), and one, with mAb 10H (3) that recognizes PAR of approximately 8 ADP-ribose units or more. The immunoprecipitated radioactivity in each sample was determined in a  $\beta$ -counter. Relative binding of modified GST-p150C to anti-PAR was determined as follows: (cpm of 3 – cpm of 2) / (cpm of 1 – cpm of 2).

**Figure 9** Autoradiogramm of a Northern blot showing ubiquitous expression of *p150*.

**Figure 10** The human *p150* gene is localized in chromosome 8q24.  
To determine the location of the human *p150* gene, FISH analysis was performed on mitotic chromosomal spreads of normal human peripheral white blood cells. The upper panel shows a complete spread and the lower panel a composite of chromosome 8 of different cells. For orientation, a schematic view of the Giemsa stain banding pattern of chromosome 8 is shown on the right.

**Figure 11** c-Myc and p150 interact in vivo and in vitro.  
A. c-Myc and p150 were expressed as tagged versions (c-myc with a FLAG-tag; p150 with an HA-tag) in COS-7 cells as indicated. F buffer lysates (Sommer, 1998) were then used for co-immunoprecipitation experiments with tag-specific antibodies. The transiently expressed proteins were analyzed on Western blots using the same tag-specific antibodies. The top two panels show the input, the bottom two panels the results of the co-immunoprecipitations. IP: immunoprecipitation; W: Western blot.  
B. In vitro translated  $^{35}\text{S}$ -labeled c-Myc (input) was incubated with GST or with GST-p150C. The proteins were then adsorbed to glutathion-agarose. Labeled c-Myc protein bound to GST or GST-p150C was recovered from glutathion-agarose, separated by SDS-PAGE and identified by autoradiography. c-Myc binds specifically to GST-p150C but not to GST demonstrating a direct interaction of c-Myc with the C-terminal portion of p150 in vitro.

**Figure 12** p150 stimulates the transactivating activity of c-Myc.

A. U2OS osteosarcoma cells were transiently transfected with the M4-mintk-luc reporter plasmid (that contains 4 Myc/Max E box binding sites (M4), a minimal tk promoter and the luciferase reporter gene) with or without a c-Myc expression plasmid and with or without expression plasmids for p150 or p150 $\Delta$ PARP as indicated. Luciferase activity was determined and standardized with co-expressed  $\beta$ -galactosidase (relative luciferase activity).

B. Transient transfections were performed as mentioned under A. In addition to the plasmids described above, plasmid expressing p110 (nucleolin) and p86 (ASH2) were co-transfected as indicated. p110 and p86 represent the two additional proteins that were originally copurified with c-Myc (see Figure 2).

**Figure 13** p150 inhibits cell growth.

A. In colony formation assays using SAOS-2 cells, the effect of p150 on cell growth was determined. The cells were co-transformed with a p150-expressing plasmid and a plasmid containing the neomycin resistance gene. Giemsa-stained colonies of geneticin-resistant transfectants were counted after 3 weeks of cultivation.

B. Primary rat embryo fibroblasts (REF) were prepared and transfected as described previously (Cerni, 1995; Austen, 1998). REF were co-transfected with plasmids expressing c-Myc, an activated form of Ha-Ras and a plasmid containing the neomycin resistance gene together with or without a plasmid encoding p150. The transfected cells were selected with geneticin, stained with Giemsa, and counted. All the cell clones had a transformed phenotype.

The following Examples further illustrate the invention.

## Material and Methods

### Cells

Jurkat cells, a human leukemic T cell line, were grown in RPMI 1640 medium containing 5% fetal calf serum, COS7 were maintained in Dulbecco's-modified Eagle medium supplemented with 10% fetal calf serum, 100 units penicillin and 100 µg/ml streptomycin. Cells were lysed in modified F-buffer (10 mM Hepes pH7.5, 50 mM NaCl, 30 mM sodium pyrophosphate, 50 mM NaF, 100 µM Na<sub>3</sub>VO<sub>4</sub>, 0.2% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 2.5 units/ml pepstatin A, 2.5 units/ml leupeptin, 0.15 mM benzamidine, 2.8 µg/ml aprotinin), incubated on ice for 10 min, vortexed for 45 sec and cleared by centrifugation at 14,000 rpm (Sommer et al., 1998).

### Chromatography

F-buffer lysates of Jurkat cells were chromatographed on Sepharose CL-6B in F-buffer. As molecular size markers, the following proteins were used: thyroglobulin 670 kDa, ferritin 440 kDa, catalase 230 kDa, aldolase 156 kDa, and bovine serum albumin 70 kDa.

### Protein sequencing

Affinity purified proteins were excised from SDS-gels, trypsinized, the fragments separated by reversed phase-HPLC, and sequenced by standard Edman degradation.

### Cloning

The full length p150 cDNA was assembled in pSPORT using the following sequences: AL040630, AL042119, AA465560, AA909139, AI335251, and AI002047 (NCBI data base accession numbers). For expression in mammalian cells, a full length p150 cDNA (KpnI-XbaI fragment) was cloned into pEVRF0-HA (a vector with a CMV promoter and an HA epitope tag) by standard techniques.

### Expression and purification of GST-p150C

For a glutathione-S-transferase (GST) fusion protein, a Smal fragment encoding amino acids 519-1025 was cloned into pGEX-4T1 (Pharmacia). The expression of GST-p150C was induced with IPTG and the cells lysed in lysis buffer (50 mM Hepes, pH 7.5, 2 mM EDTA, 150 mM NaCl, 5 mM 2-mercaptoethanol, 1% NP-40, 0.2 mM phenylmethylsulfonyl fluoride, 2.8 µg/ml aprotinin, 10 mM dithiothreitol). GST-p150C was purified on glutathione agarose beads and eluted with 5 mM glutathione in lysis buffer.

### Transient transfections and immunoprecipitation

For transient transfections, COS7 cells were plated at a density of  $8 \times 10^5$  cells per 10 cm plate and refed 2 h prior to transfection. Transfections were performed using a standard calcium phosphate protocol with 20 µg of pCMV-HA-p150 (Sommer et al., 1998). Cells were harvested 48 h after transfection in F-buffer. The expression of HA-p150 was verified by Western blotting using a mAb specific for the HA-tag. The same mAb was used to immunoprecipitate HA-p150.

### PARP assays

For PARP assays, purified GST-p150C or immunoprecipitated HA-p150 were incubated in PARP assay buffer (50 mM Tris-HCl, pH 8.0, 50 mM KCl, 10 mM MgCl<sub>2</sub>, 1 mM dithiothreitol) in the presence of 3 µCi <sup>32</sup>P-NAD<sup>+</sup> (1000 Ci/mmol) in a total volume of 20 µl at 30°C for 60 min. The reaction was stopped by adding 1 volume of 2x SDS sample buffer. Unlabeled NAD<sup>+</sup>, 3-AB, and core histones were added as indicated in the figure legends.

### Northern blotting

RNA was extracted from logarithmically growing Jurkat cells, a human leukemic T cell line, using the RNeasy total RNA kit (Qiagen). 15 µg of RNA was separated on 1% formaldehyde agarose gels. The RNA was blotted onto GeneScreen<sup>TM</sup> membrane and hybridized with a <sup>32</sup>P-random prime labeled probe (p150 cDNA) in

0.25 M NaP<sub>i</sub>, 7% SDS, 1 mM EDTA at 65°C. The membrane was washed with 50 mM NaP<sub>i</sub>, 0.5% SDS.

#### Western blotting

c-Myc and Max were detected using c-Myc-specific antibodies N-262 from Santa Cruz Biotechnology and Max-specific antiserum 85 (Cerni et al., 1995), respectively. The mAb 3F10 that recognizes the HA-tag was used for detection of HA-p150 on Western blots (Sommer et al., 1998).

#### In vitro transcription/translation

p150 was transcribed and translated in vitro from pSPORT-p150 using the TNT<sup>®</sup> kit (Promega) according to the manufacturer's specifications.

#### Preparation of p150-specific antisera

A glutathion-S-transferase (GST) fusion protein encoding the first 922 amino acids of human p150 [GST-p150(N922)] was produced in *E. coli* and two rabbits were immunized with this fusion protein using standard procedures. The sera of the two rabbits are referred to as  $\alpha$ -p150 #1 and #2.

#### **Example 1: Cloning of p150**

In order to obtain information whether Myc proteins are part of high molecular weight complexes, human Jurkat T cells were lysed in F-buffer, the proteins size fractionated and the distribution of c-Myc and Max determined. Most of c-Myc and Max migrated with a size of about 300-500 kDa suggesting that these two proteins are part of one or several multimeric complexes (Fig. 1). Very little of either protein was detected at a position where heterodimeric c-Myc/Max was expected to migrate (Fig. 1, only detectable upon prolonged exposure).

To enrich for proteins that associate with the c-Myc/Max complex, F-buffer lysates were immunoprecipitated with the monoclonal antibody (mAb) 6A10 that recognizes an N-terminal epitope of human c-Myc (Sommer et al., 1998). 50  $\mu$ g of purified mAb was covalently linked to CNBr-activated Sepharose 4B which was then used as an

affinity matrix. The proteins bound to 6A10 were compared to those bound to a matrix coupled with the isotype matched mAb 5C9 that recognized Mad1 (Sommer et al., 1997). Three proteins with apparent molecular weights of 86, 110, and 150 kDa were considered specific for the Myc-specific affinity matrix since these proteins were detected in numerous experiments but not in the controls (Fig. 2). These three proteins were excised from SDS-gels, digested with trypsin and the sequence of several peptides determined. Two of these proteins could be identified as nucleolin and Ash2, which are already known. From p150, nine different peptides were sequenced (Fig. 3), for which no matches with sequences in protein data bases were obtained. Therefore, the predicted DNA sequences were used to screen EST data bases. Several EST clones were obtained that contained some but not all of the peptides. In addition, in many instances the peptide sequences were not in the same reading frame. Thus, it was likely that some of the EST clones contained rearrangements or intronic sequences. In addition, none of the EST clones contained cDNA sequences that encoded the full length protein. Further analysis of information from data bases and comparison to murine sequences allowed to construct a cDNA with about 3.5 kb containing a large open reading frame of 1025 amino acids (Fig. 3). A full length human cDNA encoding p150 was assembled from two EST clones (AL040630 and AI002047). An EcoRI-NotI fragment from AL040630 was cloned into pSPORT1. A NotI-NotI fragment of AI002047 was then cloned into the modified pSPORT1 from the first cloning step. The 5' untranslated sequence of the cDNA was then removed using PCR cloning and a KpnI-XbaI fragment was then cloned into a CMV driven expression vector containing an HA-tag. This resulted in the generation of an HA-full length p150 fusion protein. Northern blot analysis revealed a message of about 3.7 kb indicating that a nearly full length cDNA clone was obtained (Fig. 4A). The deduced amino acid sequence contains all peptides that were identified previously. Using an *in vitro* transcription/translation approach, a protein of 150 kDa was synthesized from this cDNA supporting the notion that it encodes p150 (Fig. 4B). In addition, using fluorescence in situ hybridization, the chromosomal location of the p150 gene was assigned to chromosome 8 band q24, (Fig. 10). The q24 subband could not be determined in this experiment. This location of the p150 gene is in close proximity to the c-Myc gene which is located in this band. This close proximity is in support for functioning of the two genes in a common pathway. In Burkitt's

lymphoma, *c-myc* is translocated resulting in deregulated expression. Therefore, *Myc* gene translocations may also affect the gene encoding p150 (e.g. in Burkitt's lymphomas). Furthermore, 8q23-24 are frequently amplified in breast and prostate carcinoma, which might hint at an altered expression of *p150* in such tumors.

### Example 2: Assaying PARP activity of p150

Sequence comparison revealed a weak homology of the C-terminal 250 amino acids to the catalytic domains of poly(ADP-ribose) polymerases (Fig. 5). This sequence homology suggested that p150 may have PARP activity. Therefore, the C-terminal half of p150 (amino acids 519-1025), containing the PARP homology domain, was expressed as a glutathione-S-transferase fusion protein in *E. coli*. This protein, GST-p150C, migrated with an apparent molecular weight of about 100 kDa (Fig. 6A). In addition, several breakdown products were visible that were as abundant as the full length protein (asterisks in Fig. 6A). To determine whether bacterial GST-p150C can ADP-ribosylate proteins, GST-p150C was incubated with radioactive  $^{32}\text{P}$ -NAD $^{+}$ . A single major band was labeled corresponding to GST-p150C whereas no activity was seen with GST alone (Fig. 6B). Also none of the breakdown products was labeled suggesting that either the automodification site is localized near the C-terminus or that the modification can only occur in cis.

To test whether GST-p150C can transfer ADP-ribose from NAD $^{+}$  to other proteins, several substrates were tested. It was found that all four core histones (H2A, H2B, H3, and H4) can be ADP-ribosylated while only little label was transferred to bovine serum albumin or to the transcription factor Yin and Yang 1 (YY1; Oei, Biochem. Biophys. Res. Commun. 240 (1997), 108-111) (Fig. 6C and data not shown). This suggested that p150 encodes an enzyme that can transfer ADP-ribose from NAD $^{+}$  to itself as well as to other proteins.

One characteristic of PARP enzymes is that long ADP-ribose polymers can be synthesized. If such polymers would be formed on GST-p150C, an increase in apparent molecular weight should be detectable. Incubation of GST-p150C with  $^{32}\text{P}$ -NAD $^{+}$  in the presence of increasing amounts of unlabeled NAD $^{+}$  showed that automodified GST-p150C migrated with a decreased mobility (Fig. 6D). This is an

indication for PARP activity. However, in similar experiments with PARP1 or with tankyrase more dramatic alterations in the apparent molecular weight can be observed (Griesenbeck et al., 1997; Smith et al., 1998) suggesting that the polymers synthesized on GST-p150C may not be very long.

Further evidence that p150 catalyzes the formation of poly(ADP-ribose) (PAR) could be taken from a similar experiment as that shown in Fig. 6D, wherein the growing PAR-chains were directly detected using the antibody mAB10H that recognizes approximately 8 ADP-ribose units or more of PAR (Figure 8c).

The PARP enzymes that have been identified thus far are efficiently inhibited by 3-amino benzamide (3AB). Therefore, the sensitivity of GST-p150C to 3AB was determined (Fig. 6E). Unlike PARP1 which is inhibited efficiently with 10  $\mu$ M 3AB, GST-p150C was only inhibited at a 1000-fold higher concentration.

The biochemical analysis described above was performed with a fragment of p150 expressed in bacteria. In order to determine next whether full length p150 has PARP activity, an HA-tagged version of p150 was expressed in COS7 cells. A protein of about 150 kDa was detected in whole cell extracts of transiently transfected cells but not in vector only transfected control cells (Fig. 7A). Immunofluorescence analysis of HA-p150 showed that this protein was localized predominantly in the cytoplasm (data not shown). Incubation of immunoprecipitated HA-p150 with  $^{32}$ P-NAD<sup>+</sup> resulted in a single labeled protein species suggesting that the full length p150 also has PARP activity (Fig. 7B).

### **Example 3: Immunological characterization of p150**

In order to be able to detect and immunoprecipitate non-tagged p150, rabbit antisera  $\alpha$ -p150 #1 and #2 have been prepared by immunizing rabbits with a GST-fusion protein comprising the N-terminal 922 amino acids of p150. The specificity of these antisera for p150 has been tested in experiments the results of which are shown in Figure 8A. The data demonstrate, as shown before, that antibodies to the HA-tag recognize the tagged versions of p150. More importantly the two antisera generated against the GST-p150(N922) fusion protein but not the respective preimmune sera reacted with the anti-HA immunoprecipitated HA-tagged p150 and both sera also

immunoprecipitated HA-tagged p150. Furthermore, the two sera but neither the preimmune sera nor the anti-HA mAb immunoprecipitated a protein with an apparent molecular weight of 150 kDa. A protein with the same molecular weight was also seen in Jurkat cell lysates. The suitability of the antisera for immunoprecipitating PARP activity was examined as it is shown in Figure 8B. Antibodies to the HA-tag and both sera (#1 and #2) but not the corresponding preimmune sera immunoprecipitated PARP activity that automodified p150. In addition, sera #1 and #2 but not the respective preimmune sera immunoprecipitated PARP activity from Jurkat T cells. Thus, endogenous p150 possesses PARP activity that can be enriched by using the anti-sera generated against GST-p150(N922).

#### **Example 4: Expression analysis of p150 in human tissues**

To analyze the expression of the *p150* gene, a Northern blot analysis of RNA from different tissues was performed (the two blots were obtained from Clontech). For hybridization, a *p150* cDNA probe was used. The amount of RNA per lane is normalized with the hybridization signal of actin.

As it is shown in Fig. 9, *p150* is ubiquitously expressed with particular high levels in thymus.

#### **Example 5: Studies on the interaction between p150 and c-Myc**

In order to further substantiate the interaction between c-Myc and p150, co-immunoprecipitation experiments have been performed (Figure 11A). For this purpose, both proteins were co-expressed as differently tagged versions in COS-7 cells (c-Myc with a FLAG-tag; p150 with an HA-tag). As a control, these proteins were individually expressed. From the Western blots shown in Figure 11A, it is apparent that c-Myc can be co-immunoprecipitated from the lysate using an antibody specific for the p150-tag and vice versa. This is not the case when the proteins are expressed individually. These findings support a direct interaction of c-Myc and p150 in vivo.

Furthermore, in a pull-down assay, it could be shown that the C-terminal half of p150 is involved in this interaction (Figure 11B). Thus, the direct interaction between c-Myc and p150 could also be demonstrated in vitro.

From the data obtained on the direct binding of p150 and c-Myc, the question arose in which way p150 directly influences c-Myc function. Figure 12 documents the results of studies on the influence of p150 on c-Myc-mediated transcriptional activation (transactivation). In transient co-transfection experiments using a 4 Myc/Max E box-luciferase reporter gene construct, c-Myc-dependent transcriptional regulation can be examined. In cells being additionally co-transfected with a p150 expression plasmid, luciferase activity increased by more than two fold compared to corresponding cells without p150 expression (Figure 12A). Interestingly, expression of p150 in which the PARP domain is deleted (p150  $\Delta$  PARP) has no effect on c-Myc-dependent transactivation.

These transient co-transfection experiments were expanded in order to elucidate the influence of the other two proteins that were originally co-purified with p150 from the c-Myc complex, i.e. p110 (nucleolin) and p86 (ASH2) (Figure 12B). The results indicate that the c-Myc-dependent transactivation activity can be stimulated significantly when p150 is co-expressed with p110 and p86 but not when the PARP domain of the p150 has been deleted. The complex that is formed by c-Myc, p150, p110 and p86 that corresponds to the complex originally purified from Jurkat T cells shows the largest transactivating activity.

#### **Example 6: Effect of p150 on cell growth**

To determine the functional consequences of p150 on cell growth, colony formation assays in SAOS-2 cells (an osteosarcoma tumor cell line) were performed (Figure 13A). These cells were co-transfected with a plasmid expressing p150 and a plasmid containing the neomycin resistance gene. Stable transfectants were selected with geneticin and after 3 weeks the number of colonies was determined after Giemsa staining. Clearly, p150 expression leads to a reduction of cell growth to almost a half of that of the cells not expressing p150.

Furthermore, the potential of p150 to influence cellular transformation was investigated (Figure 13B). For this purpose, primary rat embryo fibroblasts (REF)

were co-transfected with plasmids expressing c-Myc and Ha-Ras which normally leads to their transformation (Cerni, 1995; Austen, 1998). These cells were additionally transfected with a plasmid expressing p150. As it is apparent from Figure 13 B, p150 expression abolishes c-Myc/Ha-Ras-mediated transformation of the cells. Thus, according to the assays described, the overexpression of p150 inhibits cell growth and Myc/Ras-dependent transformation. It is not quite clear how this fits with the role of p150 in the activation of c-Myc transactivation. Two possible explanations can be suggested. The first is that co-expression of p150 results in a strong stimulation of c-Myc activity which leads to the induction of apoptosis in the transfected cells. However, in preliminary experiments, it has been observed that expression of p150 blocks S phase progression (Resting 3T3L1 cells were microinjected with a plasmid encoding p150. The cells were then stimulated by adding fetal calf serum and their progression into S phase was monitored by the incorporation of BrdU as described previously (Sommer, 1997)). However, no signs of apoptosis were seen making this explanation less likely. Alternatively, and probably the more likely explanation, p150 has additional function(s) beside the stimulation of c-Myc. This or these functions are, however, not understood at present.

References

Amé, J.-C., Rolli, V., Schreiber, V., Niedergang, C., Apiou, F., Decker, P., Muller, S., Höger, T., Ménissier-de Murcia, J., and de Murcia, G. (1999). PARP-2, a novel mammalian DNA damage-dependent poly(ADP-ribose) polymerase. *J. Biol. Chem.* 274, 17860-17868.

Austen, M., Cerni, C., Lüscher-Firzlaff, J.M., and Lüscher, B. (1998), YY1 can inhibit c-Myc function through a mechanism requiring DNA binding of YY1 but neither its transactivation domain nor direct interaction with c-Myc. *Oncogene* 17, 511-520.

Burkart, V., Wang, Z. Q., Radons, J., Heller, B., Herceg, Z., Stingl, L., Wagner, E. F., and Kolb, H. (1999). Mice lacking the poly(ADP-ribose) polymerase gene are resistant to pancreatic beta-cell destruction and diabetes development induced by streptozocin. *Nat. Med.* 5, 314-319.

Cardinal, J. W., Allan, D. J., and Cameron, D. P. (1999). Poly(ADP-ribose)polymerase activation determines strain sensitivity to streptozotocin-induced beta cell death in inbred mice. *J. Mol. Endocrinol.* 22, 65-70.

Cerni, C., Bousset, K., Seelos, C., Burkhardt, H., Henriksson, M., and Lüscher, B. (1995). Differential effects by Mad and Max on transformation by cellular and viral oncoproteins. *Oncogene* 11, 587-596.

Cherney, B. W., McBride, O. W., Chen, D. F., Alkhatib, H., Bhatia, K., Hensley, P., and Smulson, M. E. (1987). cDNA sequence, protein structure, and chromosomal location of the human gene for poly(ADP-ribose) polymerase. *Proc. Natl. Acad. Sci. USA* 84, 8370-8374.

Cosi, C., and Marien, M. (1999). Implication of poly (ADP-ribose) polymerase (PARP) in neurodegeneration and brain energy metabolism. Decreases in mouse brain NAD<sup>+</sup> and ATP caused by MPTP are prevented by the PARP inhibitor benzamide. *Ann. N. Y. Acad. Sci.* 890, 227-239.

- D'Amours, D., Desnoyers, S., D'Silva, I., and Poirier, G. G. (1999). Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. *Biochem J.* 342, 249-268.
- Dang, C. V. (1999). c-Myc target genes involved in cell growth, apoptosis, and metabolism. *Mol. Cell. Biol.* 19, 1-11.
- Eliasson, M. J., Sampei, K., Mandir, A. S., Hurn, P. D., Traystman, R. J., Bao, J., Pieper, A., Wang, Z. Q., Dawson, T. M., Snyder, S. H., and Dawson, V. L. (1997). Poly(ADP-ribose) polymerase gene disruption renders mice resistant to cerebral ischemia. *Nat. Med.* 3, 1089-1095.
- Felsher, D. W., and Bishop, J. M. (1999). Transient excess of MYC activity can elicit genomic instability and tumorigenesis. *Proc. Natl. Acad. Sci. USA* 96, 3940-3944.
- Foley, K. P., and Eisenman, R. N. (1999). Two MAD tails: what the recent knockouts of Mad1 and Mxi1 tell us about the MYC/MAX/MAD network. *Biochim. Biophys. Acta.* 1423, M37-47.
- Foley, K. P., McArthur, G. A., Queva, C., Hurlin, P. J., Soriano, P., and Eisenman, R. N. (1998). Targeted disruption of the MYC antagonist MAD1 inhibits cell cycle exit during granulocyte differentiation. *EMBO J.* 17, 774-785.
- Gale, E. A. (1996a). Molecular mechanisms of beta-cell destruction in IDDM: the role of nicotinamide. *Horm. Res.* 45, 39-43.
- Gale, E. A. (1996b). Theory and practice of nicotinamide trials in pre-type 1 diabetes. *J. Pediatr. Endocrinol. Metab.* 9, 375-379.
- Griesenbeck, J., Oei, S. L., Mayer-Kuckuk, P., Ziegler, M., Buchlow, G., and Schweiger, M. (1997). Protein-protein interaction of the human poly(ADP-ribosyl)transferase depends on the functional state of the enzyme. *Biochemistry* 36, 7297-7304.

Henriksson, M., and Lüscher, B. (1996). Proteins of the Myc network: Essential regulators of cell growth and differentiation. *Adv. Cancer Res.* 68, 109-182.

Jacobson, M. K., and Jacobson, E. L. (1999). Discovering new ADP-ribose polymer cycles: protecting the genome and more. *Trends Biochem. Sci.* 24, 415-417.

Kickhoefer, V. A., Siva, A. C., Kedersha, N. L., Inman, E. M., Ruland, C., Streuli, M., and Rome, L. H. (1999). The 193-kD vault protein, VPARP, is a novel poly(ADP-ribose) polymerase. *J. Cell Biol.* 146, 917-928.

Kickhoefer, V. A., Stephen, A. G., Harrington, L., Robinson, M. O., and Rome, L. H. (1999). Vaults and telomerase share a common subunit, TEP1. *J. Biol. Chem.* 274, 32712-32717.

Kiermaier, A., and Eilers, M. (1997). Transcriptional control: Calling in histone deacetylase. *Curr. Biol.* 7, 505-507.

Koedel, U., and Pfister, H. W. (1999). Oxidative stress in bacterial meningitis. *Brain Pathol.* 9, 57-67.

Kroger, H., Miesel, R., Dietrich, A., Ohde, M., Rajnavolgyi, E., and Ockenfels, H. (1996). Synergistic effects of thalidomide and poly (ADP-ribose) polymerase inhibition on type II collagen-induced arthritis in mice. *Inflammation* 20, 203-215.

Lam, T. T. (1997). The effect of 3-aminobenzamide, an inhibitor of poly-ADP-ribose polymerase, on ischemia/reperfusion damage in rat retina. *Res. Commun. Mol. Pathol. Pharmacol.* 95, 241-252.

Love, S., Barber, R., and Wilcock, G. K. (1999). Increased poly(ADP-ribosyl)ation of nuclear proteins in Alzheimer's disease. *Brain* 122, 247-253.

- Lüscher, B., and Larsson, L.-G. (1999). The basic region/helix-loop-helix/leucine zipper domain of Myc proto-oncoproteins: Function and regulation. *Oncogene* 18, 2955-2966.
- Mandir, A. S., Przedborski, S., Jackson-Lewis, V., Wang, Z. Q., Simbulan-Rosenthal, C. M., Smulson, M. E., Hoffman, B. E., Guastella, D. B., Dawson, V. L., and Dawson, T. M. (1999). Poly(ADP-ribose) polymerase activation mediates 1-methyl-4-phenyl-1, 2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism. *Proc. Natl. Acad. Sci. USA* 96, 5774-5779.
- McArthur, G. A., Laherty, C. D., Quéva, C., Hurlin, P. J., Loo, L., James, L., Grandori, C., Gallant, P., Shiio, Y., Hokanson, W. C., Bush, A. C., Cheng, P. F., Lawrence, Q. A., Pulverer, B., Koskinen, P. J., Foley, K. P., Ayer, D. E., and Eisenman, R. N. (1998). The Mad protein family links transcriptional repression to cell differentiation. *CSH Symp. Quant. Biol. LXIII*, 423-433.
- Nagayama, T., Simon, R. P., Chen, D., Henshall, D. C., Pei, W., Stetler, R. A., and Chen, J. (2000). Activation of poly(ADP-ribose) polymerase in the rat hippocampus may contribute to cellular recovery following sublethal transient global ischemia. *J. Neurochem.* 74, 1636-1645.
- Nesbit, C. E., Tersak, J. M., and Prochownik, E. V. (1999). MYC oncogenes and human neoplastic disease. *Oncogene* 18, 3004-3016.
- Oei, S. L., and Ziegler, M. (2000). ATP for the DNA ligation step in base excision repair is generated from poly(ADP-ribose). *J. Biol. Chem.* 275, 23234-23239.
- Pieper, A. A., Brat, D. J., Krug, D. K., Watkins, C. C., Gupta, A., Blackshaw, S., Verma, A., Wang, Z. Q., and Snyder, S. H. (1999). Poly(ADP-ribose) polymerase-deficient mice are protected from streptozotocin-induced diabetes. *Proc. Natl. Acad. Sci. USA* 96, 3059-3064.

Pulverer, B., Sommer, A., McArthur, G. A., Eisenman, R. N., and Lüscher, B. (2000). Analysis of Myc/Max/Mad network members in adipogenesis: inhibition of the proliferative burst and differentiation by ectopically expressed Mad1. *J. Cell. Physiol.* 183, 399-410.

Ruf, A., Mennissier de Murcia, J., de Murcia, G., and Schulz, G. E. (1996). Structure of the catalytic fragment of poly(AD-ribose) polymerase from chicken. *Proc. Natl. Acad. Sci. USA* 93, 7481-7485.

Schreiber-Agus, N., and DePinho, R. A. (1998). Repression by the Mad(Mxi1)-Sin3 complex. *Bioessays* 20, 808-818.

Smith, S., Gariat, I., Schmitt, A., and de Lange, T. (1998). Tankyrase, a poly(ADP-ribose) polymerase at human telomeres [see comments]. *Science* 282, 1484-1487.

Sommer, A., Bousset, K., Kremmer, E., Austen, M., and Lüscher, B. (1998). Identification and characterization of specific DNA-binding complexes containing members of the Myc/Max/Mad network of transcriptional regulators. *J. Biol. Chem.* 273, 6632-6642.

Sommer, A., Hilfenhaus, S., Menkel, A., Kremmer, E., Seiser, C., Loidl, P., and Lüscher, B. (1997). Cell growth inhibition by the Mad/Max complex through recruitment of histone deacetylase activity. *Curr. Biol.* 7, 357-365.

Tsao, B. P., Cantor, R. M., Grossman, J. M., Shen, N., Teophilov, N. T., Wallace, D. J., Arnett, F. C., Hartung, K., Goldstein, R., Kalunian, K. C., Hahn, B. H., and Rotter, J. I. (1999). PARP alleles within the linked chromosomal region are associated with systemic lupus erythematosus. *J. Clin. Invest.* 103, 1135-1140.

**CLAIMS**

1. A nucleic acid molecule encoding a poly(ADP-ribose) polymerase selected from the group consisting of
  - (a) nucleic acid molecules encoding a protein which comprises the amino acid sequence indicated in SEQ ID NO: 2;
  - (b) nucleic acid molecules comprising the nucleotide sequence of the coding region indicated in SEQ ID NO: 1;
  - (c) nucleic acid molecules encoding a protein, the amino acid sequence of which has a homology of at least 30% to the amino acid sequence indicated in SEQ ID NO: 2;
  - (d) nucleic acid molecules the complementary strand of which hybridizes to a nucleic acid molecule as defined in (a) or (b); and
  - (e) nucleic acid molecules, the nucleotide sequence of which deviates because of the degeneracy of the genetic code from the sequence of the nucleic acid molecules as defined in any one of (b), (c) or (d).
2. An oligonucleotide which specifically hybridizes with the nucleic acid molecule of claim 1.
3. A vector containing the nucleic acid molecule of claim 1.
4. The vector of claim 3, wherein the nucleic acid molecule is linked to regulatory elements ensuring transcription in eukaryotic and prokaryotic cells.
5. A host cell, which is genetically modified with a nucleic acid molecule of claim 1 or with a vector of claim 3 or 4.
6. A method for the production of a protein encoded by a nucleic acid molecule of claim 1 in which the host cell of claim 5 is cultivated under conditions allowing for the expression of the protein and in which the protein is isolated from the cells and/or the culture medium.

7. A protein encoded by the nucleic acid molecule of claim 1 or obtainable by the method of claim 6.
8. An antibody specifically recognizing the protein of claim 7.
9. A pharmaceutical composition comprising the protein of claim 7 or a nucleic acid molecule of claim 1 and optionally a pharmaceutically acceptable carrier or excipient.
10. Use of the protein of claim 7 or of the nucleic acid molecule of claim 1 for the preparation of a pharmaceutical composition for the prevention and/or treatment of tumors.
11. An antagonist of the protein of claim 7.
12. A pharmaceutical composition comprising the antagonist of claim 11 and optionally a pharmaceutically acceptable carrier or excipient.
13. Use of the antagonist of claim 11 for the preparation of a pharmaceutical composition for the treatment of cerebral ischaemia, diabetes, Alzheimer's disease, Parkinson's disease, systemic lupus erythromatosus (SLE) or arthritis or for the prevention and/or treatment of tumors.
14. A diagnostic composition comprising the nucleic acid molecule of claim 1, the oligonucleotide of claim 2, the protein of claim 7 and/or the antibody of claim 8.
15. A method for screening compounds to identify those which act as agonists or antagonists of the protein of claim 7, comprising the steps of
  - (a) measuring the ADP-ribosylating activity of a protein of claim 7 in the presence and in the absence of the compound to be tested; and
  - (b) determining that the activity measured in step (a) is
    - (i) higher in the presence of the compound than in the absence of the compound, thereby identifying a compound acting as agonist; or

(ii) lower in the presence of the compound than in the absence of the compound, thereby identifying a compound acting as antagonist.

16. A method for preparing a pharmaceutical composition comprising the steps of identifying an agonist or an antagonist of the protein of claim 7 by one of the methods of claim 15 or 16 and formulating the identified agonist or antagonist in a pharmaceutical composition.

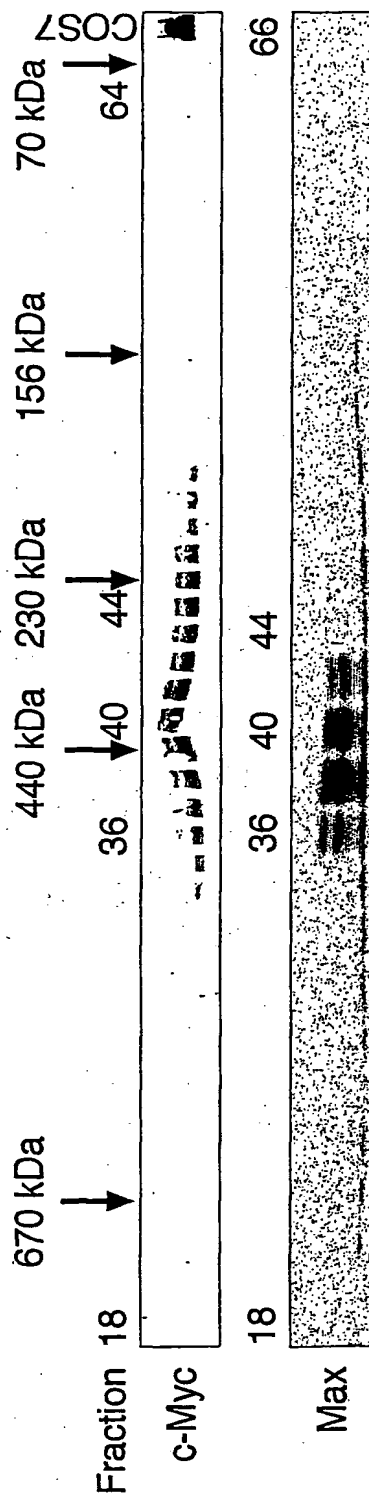


Figure 1  
Lüscher and Austen

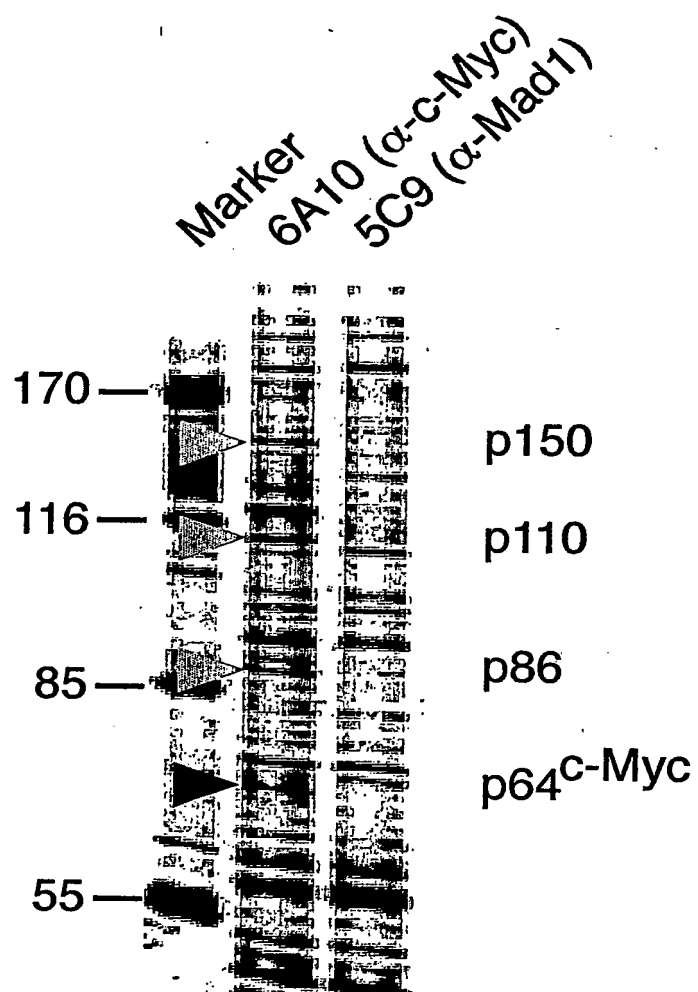


Figure 2  
Lüscher and Austen

Figure 3  
Lüscher and Austen

## Sequence of pl50

GGT ACG CCT GCA GGT ACC GGT CCG GAA TTC CCG GGT CGA CCC ACG CGT CCG CTT GCT GAG T  
 GGG CAG GCA CAG GAG TCC TGG CTG AGC TCA TGG CCT GAG GCT GCC TAG CGG CCA CGG GGA  
 1/1 31/11  
 ATG GTT GCA ATG GCG GAG GCA GAG GCA GGG GTG GCA GTG GAG GTC CGT GGA CTG CCC CCT  
 M V A M A E A E A G V A V E V R G L P P T70  
 61/21 91/31  
 GCC GTG CCC GAC GAG CTG CTC ACT CTC TAC TTT GAA AAC CGC CGA CGC TCT GGA GGG GGA  
 A V P D E L L T L Y F E N R R R S G G G  
 121/41 151/51  
 CCT GTG TTG AGC TGG CAG AGA CTG GGC TGT GGG GGC GTC CTC ACC TTC AGA GAG CCT GCA  
 P V L S W Q R L G C G G V L T F R E P A  
 181/61 211/71  
 GAC GCC GAG AGG GTC TTG GCC CAG GCA GAT CAC GAA CTA CAT GGT GCC CAG CTG AGC CTG  
 D A E R V L A Q A D H E L H G A Q L S L  
 241/81 271/91  
 CGG CCA GCT CCA CCA CGA GCC CCT GCA CGC CTG CTG CTC CAA GGA CTG CCC CCT GGC ACC  
 R P A P P R A P A R L L L Q G L P P G T T65  
 301/101 331/111  
 ACG CCC CAG CGC TTG GAG CAG CAT GTC CAG GCC TTG CTG CGG GCC TCG GGG CTC CCA GTA  
 T P Q R L E Q H V Q A L L R A S G L P V  
 361/121 391/131  
 CAG CCT TGC TGT GCC TTG GCC AGC CCC CGG CCA GAC CGG GCT CTG GTC CAG TTG CCC AAG  
 Q P C C A L A S P R P D R A L V Q L P K  
 421/141 451/151  
 CCC CTT TCT GAG GCA GAT GTC CGT GTC CTG GAG GAG CAG GCC CAG AAT CTG GGC CTG GAG  
 P L S E A D V R V L E E Q A Q N L G L E T59  
 481/161 511/171  
 GGG ACC TTG GTG TCC CTG GCC CGG GTT CCG CAG GCC CGA GCG GTG CGT GTG GTG GGG GAT  
 G T L V S L A R V P Q A R A V R V V G D  
 541/181 571/191  
 GGT GCC TCT GTG GAC CTG CTG TTG CTG GAG TTG TAC CTG GAG AAT GAG CGC CGC AGT GGT  
 G A S V D L L L L E L Y L E N E R R S G  
 601/201 631/211  
 GGG GGG CCC CTG GAG GAC CTG CAA CGC CTA CCC GGG CCC CTG GGC ACT GTT GCC TCC TTC  
 G G P L E D L Q R L P G P L G T V A S F  
 661/221 691/231  
 CAG CAG TGG CAA GTG GCA GAA CGA GTG TTG CAG CAG GAG CAC CGG TTG CAG GGC TCA GAG  
 Q Q W Q V A E R V L Q Q E H R L Q G S E T57  
 721/241 751/251  
 CTG AGC CTT GTC CCC CAC TAC GAC GTC CTG GAG CCC GAG GAG CTG GCT GAG AAC ACC AGT  
 L S L V P H Y D V L E P E E L A E N T S  
 781/261 811/271  
 GGA GGG GAC CAC CCG TCC ACC CAG GGG CCT AGG GCT ACC AAG CAT GCT CTC CTG AGG ACC  
 G G D H P S T Q G P R A T K H A L L R T T44  
 841/281 871/291  
 GGA GGG TTG GTG ACG GCT CTG CAG GGT GCA GGG ACT GTG ACA ATG GGC TCT GGC GAG GAA  
 G G L V T A L Q G A G T V T M G S G E E

901/301  
CCA GGG CAG TCA GGG GCC TCT CTG AGG ACA GGT CCC ATG GTG CAG GGT AGA GGG ATT ATG  
P G Q S G A S L R T G P M V Q G R G I M

931/311  
961/321  
ACA ACA GGC TCT GGC CAG GAA CCA GGG CAG TCA GGG ACC TCT CTG AGG ACA GGT CCC ATG  
T T G S G Q E P G Q S G T S L R T G P M

991/331  
1021/341  
GGG TCT CTG GGA CAG GCA GAG CAA GTC AGC TCG ATG CCC ATG GGG TCT CTG GAA CAT GAG  
G S L G Q A E Q V S S M P M G S L E H E

1051/351  
1081/361  
GGG CTG GTA AGC CTG AGG CCT GTG GGG TTG CAG GAA CAG GAG GGG CCC ATG AGC CTG GGG  
G L V S L R P V G L Q E Q E G P M S L G

1111/371  
1141/381  
CCT GTG GGG TCT GCA GGC CCA GTG GAG ACC TCT AAG GGG TTG CTG GGG CAG GAG GGC CTG  
P V G S A G P V E T S K G L L G Q E G L

1171/391  
1201/401  
GTG GAA ATT GCC ATG GAC TCA CCA GAG CAA GAG GGG CTG GTG GGT CCC ATG GAG ATC ACC  
V E I A M D S P E Q E G L V G P M E I T

1231/411  
1261/421  
ATG GGG TCT CTG GAG AAG GCA GGG CCT GTG AGC CCA GGA TGT GTG AAG CTG GCA GGG CAG  
M G S L E K A G P V S P G C V K L A G Q

1291/431  
1321/441  
GAG GGC CTG GTG GAG ATG GTG CTA TTG ATG GAG CCA GGG GCG ATG CGC TTC CTG CAG CTC  
E G L V E M V L L M E P G A M R F L Q L

1351/451  
1381/461  
TAC CAT GAG GAC CTT CTT GCG GGC CTG GGA GAC GTC GCT CTC TTG CCA CTT GAA GGA CCG  
Y H E D L L A G L G D V A L L P L E G P

1411/471  
1441/481  
GAT ATG ACT GGC TTT CGG CTC TGT GGA GCC CAG GCT TCC TGC CAG GCG GCT GAG GAG TTT  
D M T G F R L C G A Q A S C Q A A E E F

1471/491  
1501/501  
CTG CGG AGC CTG CTG GGC AGC ATT AGC TGC CAT GTG TTG TGC CTG GAG CAC CCG GGC AGC  
L R S L L G S I S C H V L C L E H P G S

1531/511  
1561/521  
GCC AGG TTT CTC CTG GGC CCA GAA GGG CAG CAC CTT CTC CAG GGG CTG GAG GCT CAG TTC  
A R F L L G P E G Q H L L Q G L E A Q F

1591/531  
1621/541  
CAG TGT GTC TTT GGG ACA GAG CGC CTG GCC ACA GCC ACG TTG GAC ACA GGC CTT GAA GAG  
Q C V F G T E R L A T A T L D T G L E E

1651/551  
1681/561  
GTG GAC CCT ACC GAG GCC CTC CCA GTG CTC CCT GGC AAC GCC CAC ACC CTG TGG ACC CCA  
V D P T E A L P V L P G N A H T L W T P

1711/571  
1741/581  
GAC AGT ACA GGT GGT GAC CAG GAG GAC GTG AGC CTG GAG GAG GTC CGA GAA CTG CTG GCC  
D S T G G D Q E D V S L E E V R E L L A

1771/591  
1801/601  
ACC CTG GAG GGC CTA GAC CTA GAC GGG GAG GAC TGG CTG CCT CGG GAG CTG GAG GAG GAA  
T L E G L D L D G E D W L P R E L E E

1831/611

T52

5/16

Figure 3 cont.

1861/621

GGG CCT CAG GAG CAG CCA GAG GAG GAG GTG ACC CCA GGG CAT GAG GAG GAG GAG CCT GTG  
G P Q E Q P E E E V T P G H E E E E P V

1891/631

1921/641

GCC CCC AGC ACT GTG GCA CCC AGG TGG CTG GAG GAG GAG GCC GCT CTG CAG CTG GCC CTC  
A P S T V A P R W L E E E A A L Q L A L

1951/651

1981/661

CAC CGG TCA CTG GAG CCT CAA GGT CAG GTG GCT GAG CAG GAG GAG GCT GCT GCC CTG CGG  
H R S L E P Q G Q V A E Q E E A A A L R

2011/671

2041/681

CAA GCC CTA ACC CTC TCC CTG CTG GAG CAG CCC CCG TTG GAG GCA GAA GAA CCC CCA GAT  
Q A L T L S L L E Q P P L E A E E P P D

2071/691

2101/701

GGG GGG ACT GAT GGC AAG GCC CAG CTG GTG GTG CAC TCG GCC TTT GAG CAG GAT GTG GAG  
G G T D G K A Q L V V H S A F E Q D V E

2131/711

2161/721

GAG CTG GAC CGG GCG CTC AGG GCT GCC TTG GAG GTC CAC GTC CAG GAG GAG ACG GTG GGG  
E L D R A L R A A L E V H V Q E E T V G

2191/731

2221/741

CCC TGG CGC CGC ACA CTG CCT GCA GAG CTG CGT GCT CGC CTG GAG CGG TGC CAT GGT GTG  
P W R R T L P A E L R A R L E R C H G V

2251/751

2281/761

AGT GTT GCC CTG CGT GGT GAC TGC ACC ATC CTC CGT GGC TTC GGG GCC CAC CCT GCC CGT  
S V A L R G D C T I L R G F G A H P A R

2311/771

2341/781

GCT GCC CGC CAC TTG GTG GCA CTT CTG GCT GGC CCC TGG GAT CAG AGT TTG GCC TTT CCC  
A A R H L V A L L A G P W D Q S L A F P

2371/791

2401/801

TTG GCA GCT TCA GGC CCT ACC TTG GCG GGG CAG ACG CTG AAG GGG CCC TGG AAC AAC CTG  
L A A S G P T L A G Q T L K G P W N N L

2431/811

2461/821

GAG CGT CTG GCA GAG AAC ACC GGG GAG TTC CAG GAG GTG GTG CGG GCC TTC TAC GAC ACC  
E R L A E N T G E F Q E V V R A F Y D T

2491/831

2521/841

CTG GAC GCT GCC CGC AGC AGC ATC CGC GTC GTT CGT GTG GAG CGC GTG TCG CAC CCG CTG  
L D A A R S S I R V V R V E R V S H P L

2551/851

2581/861

CTG CAG CAG CAG TAT GAG CTG TAC CGG GAG CGC CTG CTG CAG CGA TGC GAG CGG CGC CCG  
L Q Q Q Y E L Y R E R L L Q R C E R R P

2611/871

2641/881

GTG GAG CAG GTG CTG TAC CAC GGC ACG ACG GCA CCG GCA GTG CCT GAC ATC TGC GCC CAC  
V E Q V L Y H G T T A P A V P D I C A H

2671/891

2701/901

GGC TTC AAC CGC AGC TTC TGC GGC CGC AAC GCC ACG GTC TAC GGG AAG GGC GTG TAT TTC  
G F N R S F C G R N A T V Y G K G V Y F

2731/911

2761/921

GCC AGG CGC GCC TCC CTG TCG GTG CAG GAC CGC TAC TCG CCC CCC AAC GCC GAT GGC CAT  
A R R A S L S V Q D R Y S P P N A D G H

2791/931

T39

T34

6/16

Figure 3 cont.

2821/941

AAG GCG GTG TTC GTG GCA CGG GTG CTG ACT GGC GAC TAC GGG CAG GGC CGC CGC GGT CTG  
K A V F V A R V L T G D Y G Q G R R G L

T28

2881/961

CGG GCG CCC CCT CTG CGG GGT CCT GGC CAC GTG CTC CTG CGC TAC GAC AGC GCC GTG GAC  
R A P P L R G P G H V L L R Y D S A V D

2911/971

2941/981

TGC ATC TGC CAG CCC AGC ATC TTC GTC ATC TTC CAC GAC ACC CAG GCG CTG CCC ACC CAC  
C I C Q P S I F V I F H D T Q A L P T H

2971/991

3001/1001

CTC ATC ACC TGC GAG CAC GTG CCC CGC GCT TCC CCC GAC GAC CCC TCT GGG CTC CCG GGC  
L I T C E H V P R A S P D D P S G L P G

3031/1011

3061/1021

CGC TCC CCA GAC ACT TAA CCG AAG GGG CCA CCC TCT GGC CTC CTG CTT CCC AGG CTC CCA  
R S P D T \*

GCT CCG CAC AGG CTG ATG CTC CCC GCC CCC AAC TGT GGC CGC CTG AGC TGT CCC CGG GGA  
CGC CCC TGC CTC CCT CTG CGG GCT CCA GAA GGC GGT GTG GGG GAT GGC GGT CAG CAG CGG  
CCG AGA GGG GCT GGG CTA GGT CCC AGC CTG GGC CGA CCC CAC CAC CAG GGG TCA GCA GAG  
CCC AGG AGG CGA CAC TGC CCG CCC GCC GCT CCC AGA CCT CGC CCG AGT CGG CTC TGT TGT  
TTG AAT AAA CGT GAA CGT GAA CCC AGG CGG AAG GGA CCC GGG AAA AAA AAA AAA AAA AAA

A



B

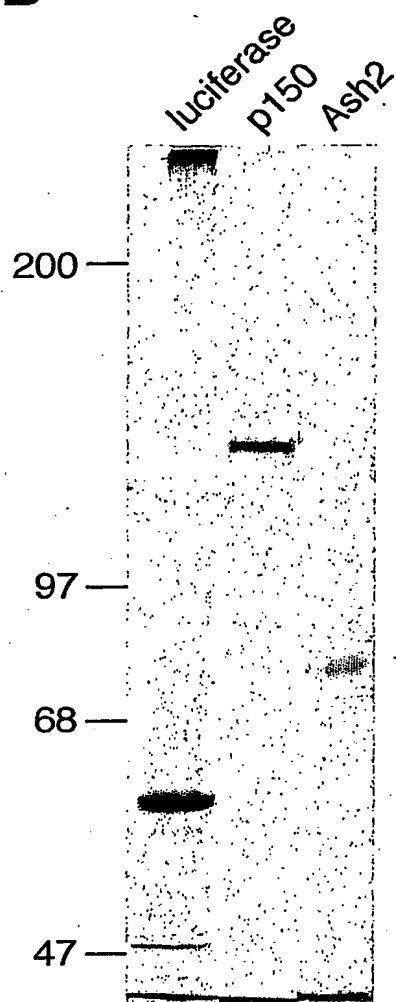


Figure 4  
Lüscher and Austen

8/16

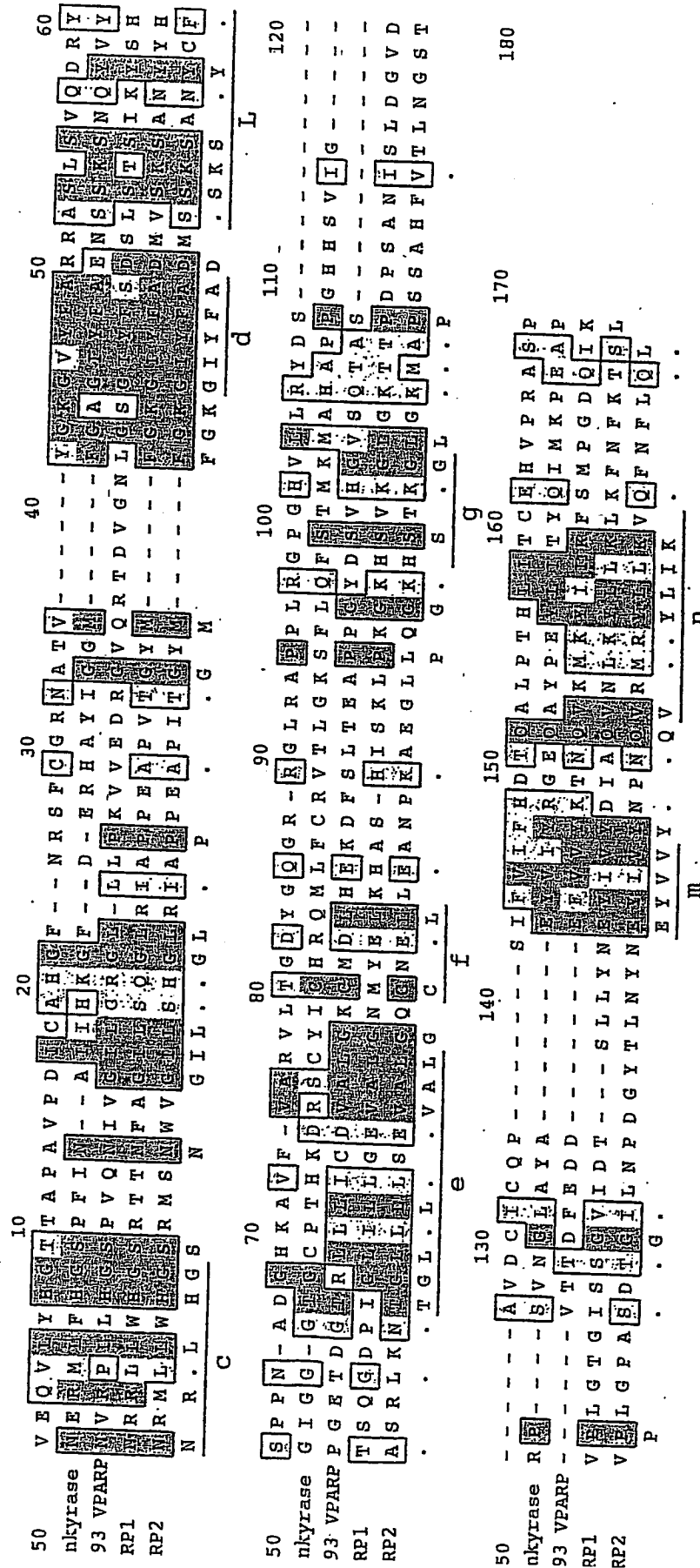


Figure 5  
Lüscher and Austen

9/16

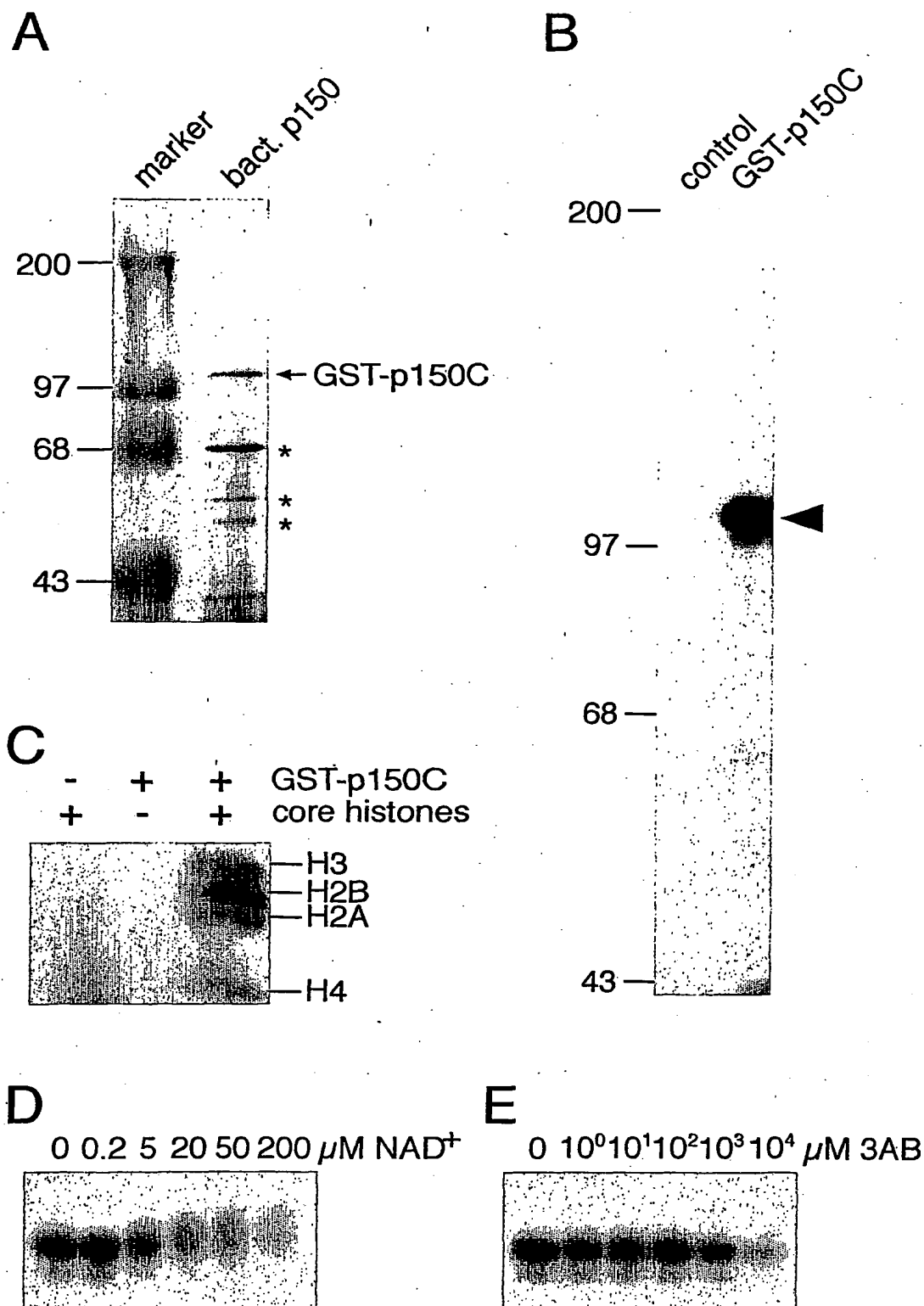


Figure 6  
Lüscher and Austen

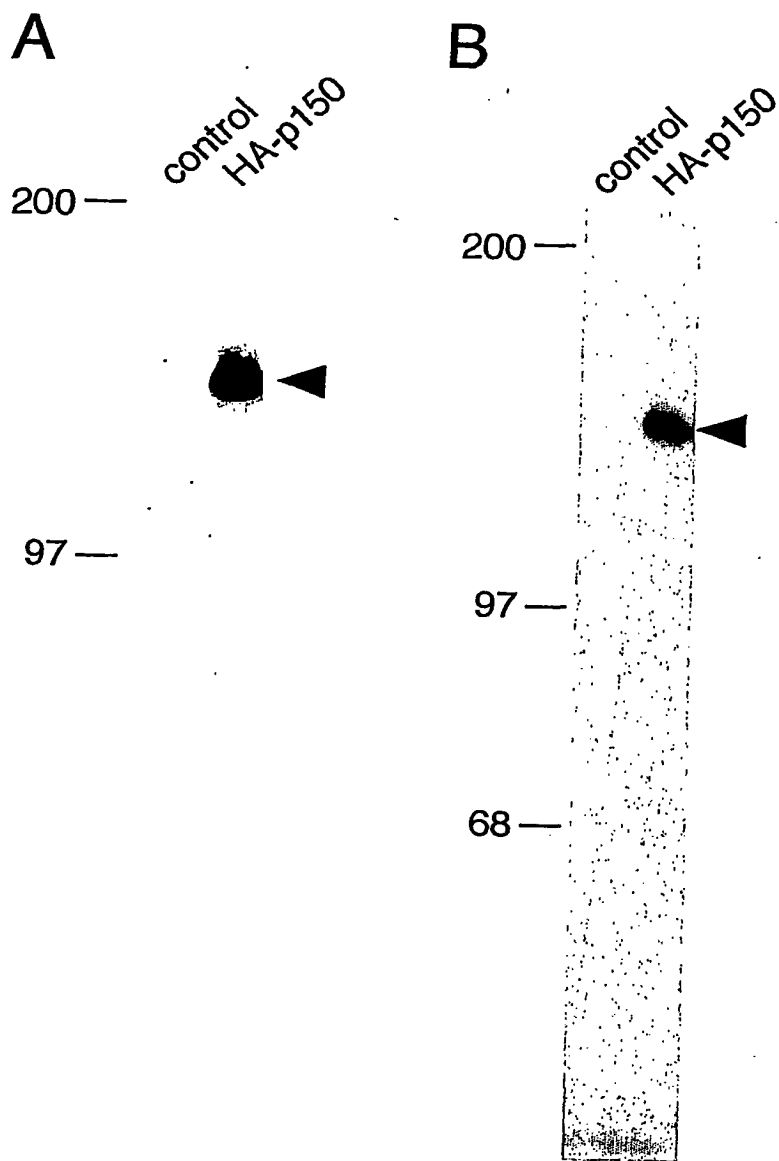
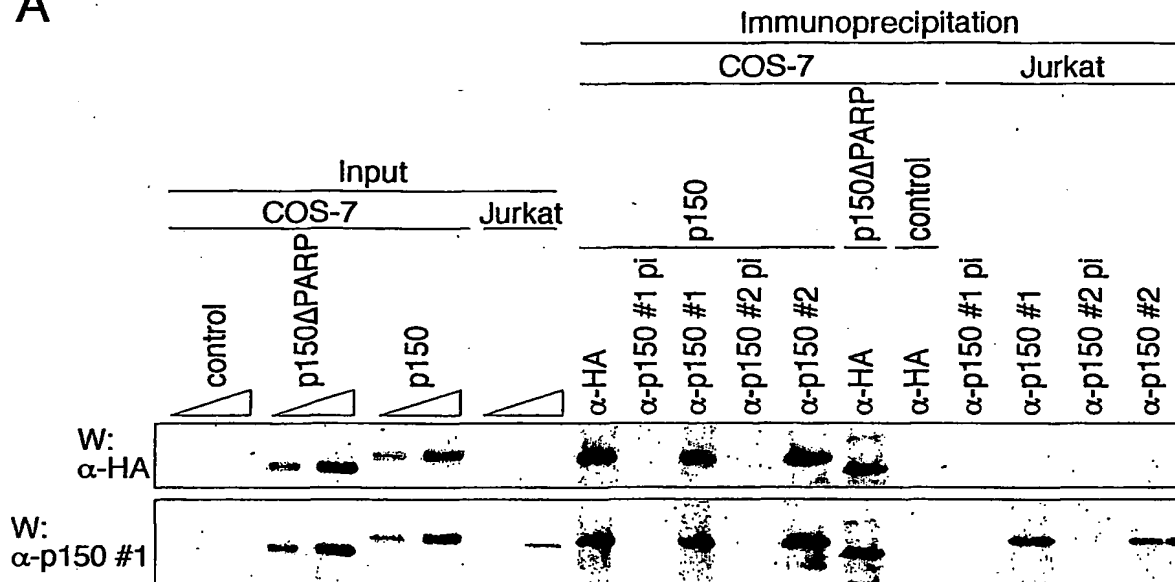


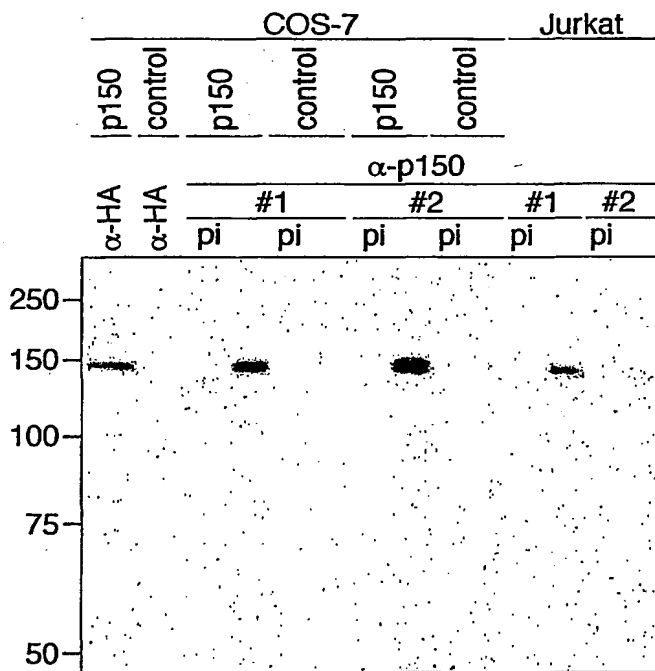
Figure 7  
Lüscher and Austen

11/16

A



B



C

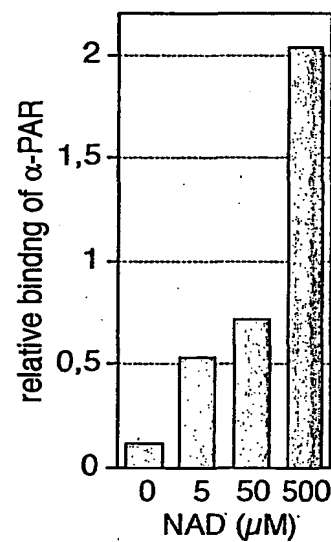


Figure 8  
Lüscher and Austen

12/16

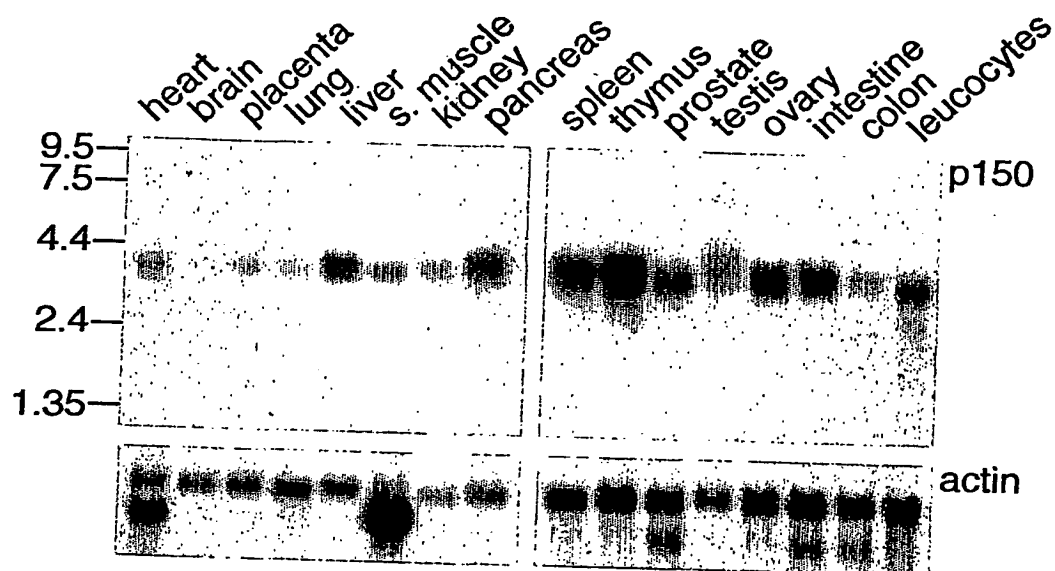


Figure 9  
Lüscher and Austen

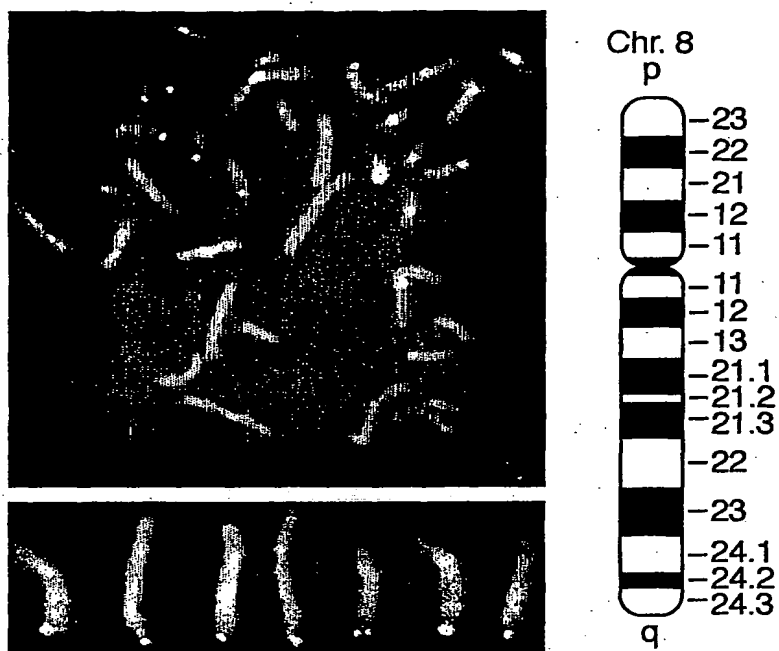


Figure 10  
Lüscher and Austen

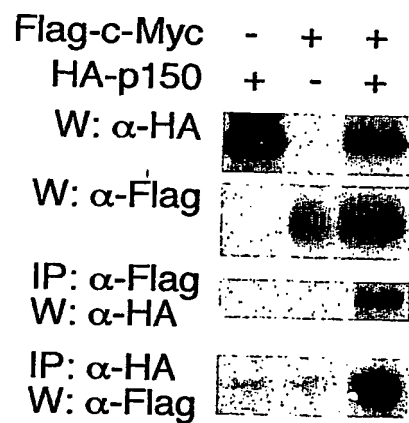
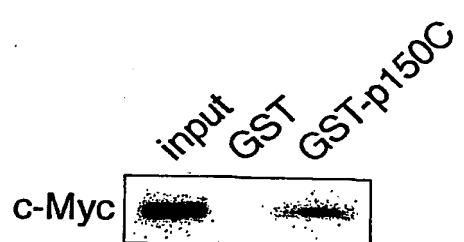
**A****B**

Figure 11  
Lüscher and Austen

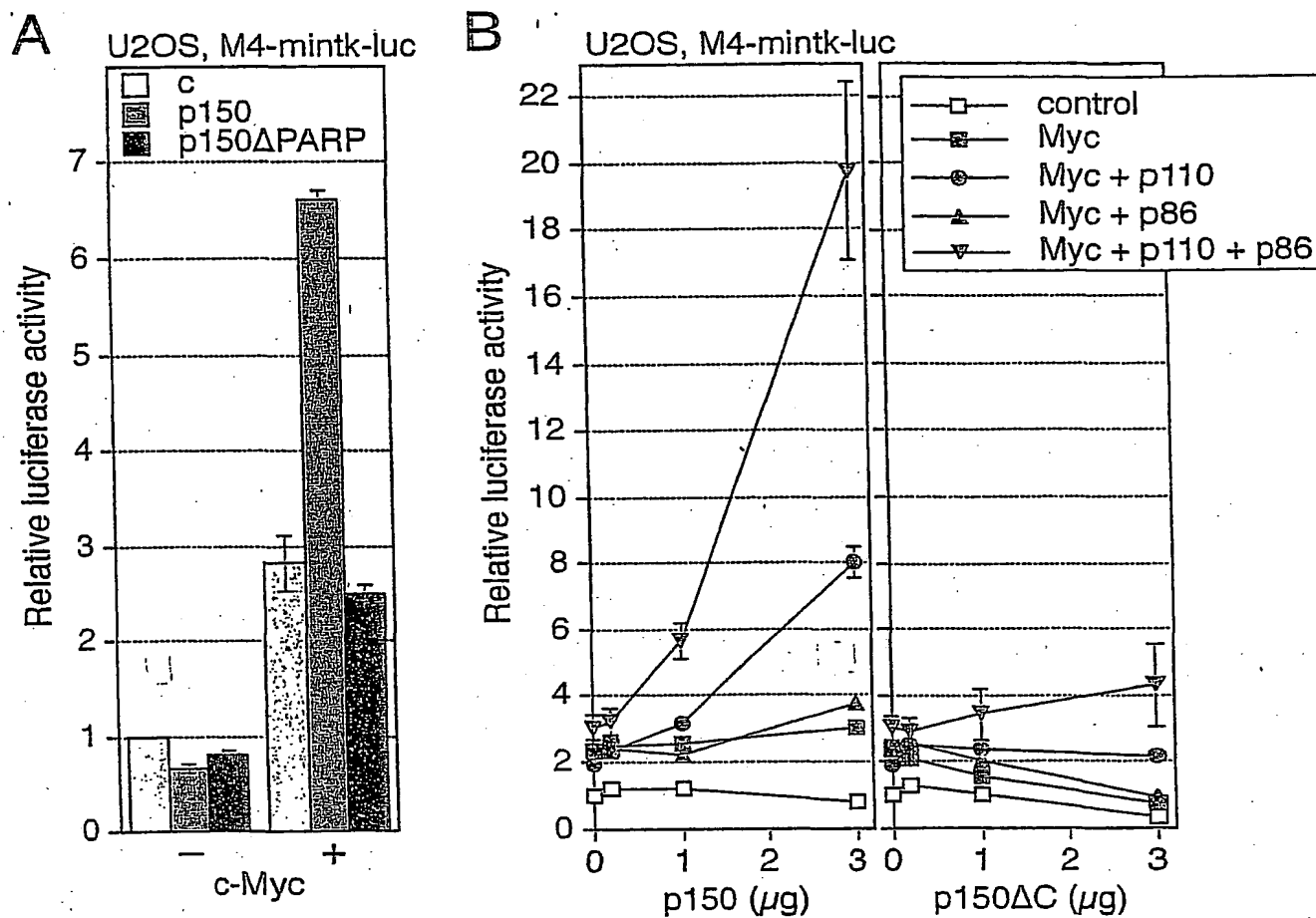


Figure 12  
Lüscher and Austen

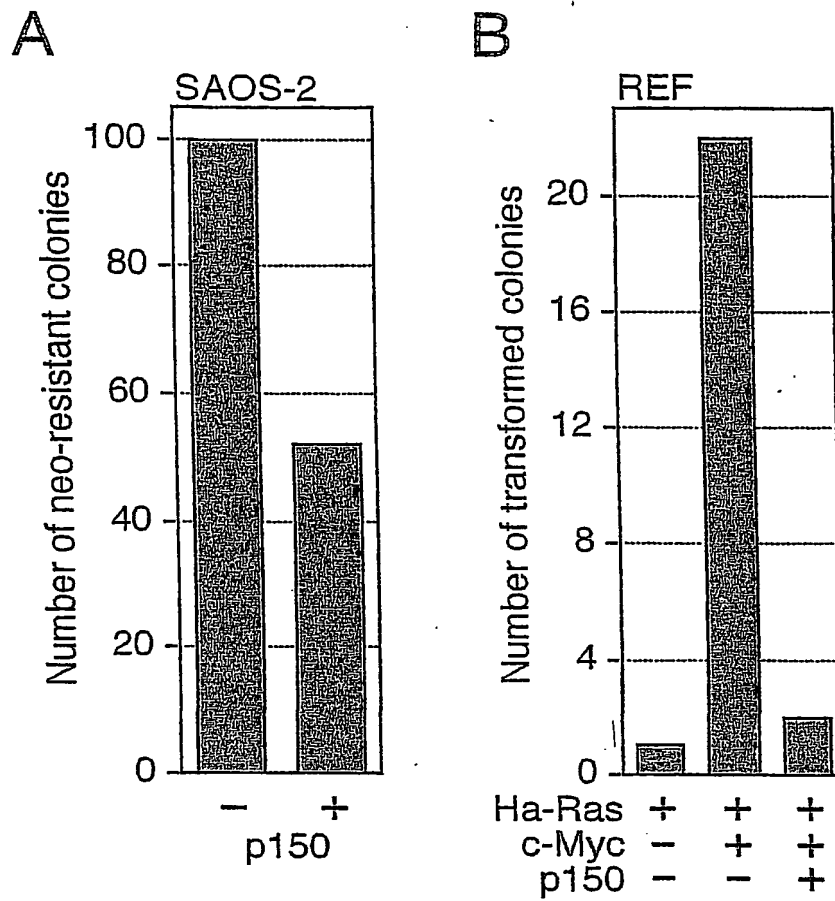


Figure 13  
Lüscher and Austen

## SEQUENCE LISTING

<110> LÜSCHER, Bernhard  
AUSTEN, Matthias

<120> Nucleic acid molecules encoding a poly (ADP-ribose)  
polymerase

<130> E 2206 PCT

<140>

<141>

<160> 2

<170> PatentIn Ver. 2.1

<210> 1

<211> 3541

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (122) .. (3196)

<400> 1

```

tggtacgcct gcaggtaccg gtccggaatt cccgggtcga cccacgcgtc cgcttgetga 60
ggggcaggca caggagtcct ggctgagctc atggcctgag gctgcctagc ggccacgggg 120
a atg gtt gca atg gcg gag gca gag gca ggg gtg gca gtg gag gtc cgt 169
  Met Val Ala Met Ala Glu Ala Glu Ala Gly Val Ala Val Glu Val Arg
    1             5             10             15

gga ctg ccc cct gcc gtg ccc gac gag ctg ctc act ctc tac ttt gaa 217
Gly Leu Pro Pro Ala Val Pro Asp Glu Leu Leu Thr Leu Tyr Phe Glu
      20             25             30

aac cgc cga cgc tct gga ggg gga cct gtg ttg agc tgg cag aga ctg 265
Asn Arg Arg Arg Ser Gly Gly Gly Pro Val Leu Ser Trp Gln Arg Leu
      35             40             45

ggc tgt ggg ggc gtc ctc acc ttc aga gag cct gca gac gcc gag agg 313
Gly Cys Gly Gly Val Leu Thr Phe Arg Glu Pro Ala Asp Ala Glu Arg
      50             55             60

gtc ttg gcc cag gca gat cac gaa cta cat ggt gcc cag ctg agc ctg 361
Val Leu Ala Gln Ala Asp His Glu Leu His Gly Ala Gln Leu Ser Leu
      65             70             75             80

cgg cca gct cca cca cga gcc cct gca cgc ctg ctg ctc caa gga ctg 409
Arg Pro Ala Pro Pro Arg Ala Pro Ala Arg Leu Leu Leu Gln Gly Leu
      85             90             95

ccc cct ggc acc acg ccc cag cgc ttg gag cag cat gtc cag gcc ttg 457
Pro Pro Gly Thr Thr Pro Gln Arg Leu Glu Gln His Val Gln Ala Leu
      100             105             110

ctg cgg gcc tcg ggg ctc cca gta cag cct tgc tgt gcc ttg gcc agc 505
Leu Arg Ala Ser Gly Leu Pro Val Gln Pro Cys Cys Ala Leu Ala Ser
      115             120             125

```

ccc cgg cca gac cgg gct ctg gtc cag ttg ccc aag ccc ctt tct gag	553
Pro Arg Pro Asp Arg Ala Leu Val Gln Leu Pro Lys Pro Leu Ser Glu	
130 135 140	
gca gat gtc cgt gtc ctg gag gag cag gcc cag aat ctg ggc ctg gag	601
Ala Asp Val Arg Val Leu Glu Glu Gln Ala Gln Asn Leu Gly Leu Glu	
145 150 155 160	
ggg acc ttg gtg tcc ctg gcc cgg gtt ccc cag gcc cga gcg gtg cgt	649
Gly Thr Leu Val Ser Leu Ala Arg Val Pro Gln Ala Arg Ala Val Arg	
165 170 175	
gtg gtg ggg gat ggt gcc tct gtg gac ctg ctg ttg ctg gag ttg tac	697
Val Val Gly Asp Gly Ala Ser Val Asp Leu Leu Leu Leu Glu Leu Tyr	
180 185 190	
ctg gag aat gag cgc cgc agt ggt ggg ggg ccc ctg gag gac ctg caa	745
Leu Glu Asn Glu Arg Arg Ser Gly Gly Gly Pro Leu Glu Asp Leu Gln	
195 200 205	
cgc cta ccc ggg ccc ctg ggc act gtt gcc tcc ttc cag cag tgg caa	793
Arg Leu Pro Gly Pro Leu Gly Thr Val Ala Ser Phe Gln Gln Trp Gln	
210 215 220	
gtg gca gaa cga gtg ttg cag cag gag cac cgg ttg cag ggc tca gag	841
Val Ala Glu Arg Val Leu Gln Gln Glu His Arg Leu Gln Gly Ser Glu	
225 230 235 240	
ctg agc ctt gtc ccc cac tac gac gtc ctg gag ccc gag gag ctg gct	889
Leu Ser Leu Val Pro His Tyr Asp Val Leu Glu Pro Glu Glu Leu Ala	
245 250 255	
gag aac acc agt gga ggg gac cac ccg tcc acc cag ggg cct agg gct	937
Glu Asn Thr Ser Gly Gly Asp His Pro Ser Thr Gln Gly Pro Arg Ala	
260 265 270	
acc aag cat gct ctc ctg agg acc gga ggg ttg gtg acg gct ctg cag	985
Thr Lys His Ala Leu Leu Arg Thr Gly Gly Leu Val Thr Ala Leu Gln	
275 280 285	
ggt gca ggg act gtg aca atg ggc tct ggc gag gaa cca ggg cag tca	1033
Gly Ala Gly Thr Val Thr Met Gly Ser Gly Glu Gln Pro Gly Gln Ser	
290 295 300	
ggg gcc tct ctg agg aca ggt ccc atg gtg cag ggt aga ggg att atg	1081
Gly Ala Ser Leu Arg Thr Gly Pro Met Val Gln Gly Arg Gly Ile Met	
305 310 315 320	
aca aca ggc tct ggc cag gaa cca ggg cag tca ggg acc tct ctg agg	1129
Thr Thr Gly Ser Gly Gln Glu Pro Gly Gln Ser Gly Thr Ser Leu Arg	
325 330 335	
aca ggt ccc atg ggg tct ctg gga cag gca gag caa gtc agc tcg atg	1177
Thr Gly Pro Met Gly Ser Leu Gly Gln Ala Glu Gln Val Ser Ser Met	
340 345 350	
ccc atg ggg tct ctg gaa cat gag ggg ctg gta agc ctg agg cct gtg	1225
Pro Met Gly Ser Leu Glu His Glu Gly Leu Val Ser Leu Arg Pro Val	
355 360 365	
ggg ttg cag gaa cag gag ggg ccc atg agc ctg ggg cct gtg ggg tct	1273
Gly Leu Gln Glu Gln Glu Gly Pro Met Ser Leu Gly Pro Val Gly Ser	
370 375 380	

gca ggc cca gtg gag acc tct aag ggg ttg ctg ggg cag gag ggc ctg	1321
Ala Gly Pro Val Glu Thr Ser Lys Gly Leu Leu Gly Gln Glu Gly Leu	
385 390 395 400	
gtg gaa att gcc atg gac tca cca gag caa gag ggg ctg gtg ggt ccc	1369
Val Glu Ile Ala Met Asp Ser Pro Glu Gln Glu Gly Leu Val Gly Pro	
405 410 415	
atg gag atc acc atg ggg tct ctg gag aag gca ggg cct gtg agc cca	1417
Met Glu Ile Thr Met Gly Ser Leu Glu Lys Ala Gly Pro Val Ser Pro	
420 425 430	
gga tgt gtg aag ctg gca ggg cag gag ggc ctg gtg gag atg gtg cta	1465
Gly Cys Val Lys Leu Ala Gly Gln Glu Gly Leu Val Glu Met Val Leu	
435 440 445	
ttg atg gag cca ggg gcg atg cgc ttc ctg cag ctc tac cat gag gac	1513
Leu Met Glu Pro Gly Ala Met Arg Phe Leu Gln Leu Tyr His Glu Asp	
450 455 460	
ctt ctt gcg ggc ctg gga gac gtc gct ctc ttg cca ctt gaa gga ccg	1561
Leu Leu Ala Gly Leu Gly Asp Val Ala Leu Leu Pro Leu Glu Gly Pro	
465 470 475 480	
gat atg act ggc ttt cgg ctc tgt gga gcc cag gct tcc tgc cag gcg	1609
Asp Met Thr Gly Phe Arg Leu Cys Gly Ala Gln Ala Ser Cys Gln Ala	
485 490 495	
gct gag gag ttt ctg cgg agc ctg ctg ggc agc att agc tgc cat gtg	1657
Ala Glu Glu Phe Leu Arg Ser Leu Leu Gly Ser Ile Ser Cys His Val	
500 505 510	
ttg tgc ctg gag cac ccg ggc agc gcc agg ttt ctc ctg ggc cca gaa	1705
Leu Cys Leu Glu His Pro Gly Ser Ala Arg Phe Leu Leu Gly Pro Glu	
515 520 525	
ggg cag cac ctt ctc cag ggg ctg gag gct cag ttc cag tgt gtc ttt	1753
Gly Gln His Leu Leu Gln Gly Leu Glu Ala Gln Phe Gln Cys Val Phe	
530 535 540	
ggg aca gag cgc ctg gcc aca gcc acg ttg gac aca ggc ctt gaa gag	1801
Gly Thr Glu Arg Leu Ala Thr Ala Thr Leu Asp Thr Gly Leu Glu Glu	
545 550 555 560	
gtg gac cct acc gag gcc ctc cca gtg ctc cct ggc aac gcc cac acc	1849
Val Asp Pro Thr Glu Ala Leu Pro Val Leu Pro Gly Asn Ala His Thr	
565 570 575	
ctg tgg acc cca gac agt aca ggt ggt gac cag gag gac gtg agc ctg	1897
Leu Trp Thr Pro Asp Ser Thr Gly Gly Asp Gln Glu Asp Val Ser Leu	
580 585 590	
gag gag gtc cga gaa ctg ctg gcc acc ctg gag ggc cta gac cta gac	1945
Glu Glu Val Arg Glu Leu Leu Ala Thr Leu Glu Gly Leu Asp Leu Asp	
595 600 605	
ggg gag gac tgg ctg cct cgg gag ctg gag gag gaa ggg cct cag gag	1993
Gly Glu Asp Trp Leu Pro Arg Glu Leu Glu Glu Glu Gly Pro Gln Glu	
610 615 620	
cag cca gag gag gag gtg acc cca ggg cat gag gag gag gag cct gtg	2041
Gln Pro Glu Glu Glu Val Thr Pro Gly His Glu Glu Glu Glu Pro Val	

625		630		635		640	
gcc ccc agc act	gtg gca ccc agg	tgg ctg gag gag gag	gcc gct ctg	2089			
Ala Pro Ser Thr	Val Ala Pro Arg Trp	Leu Glu Glu Glu Ala Ala Leu					
	645	650	655				
cag ctg gcc ctc	cac cgg tca ctg	gag cct caa ggt cag	gtg gct gag	2137			
Gln Leu Ala Leu	His Arg Ser Leu	Glu Pro Gln Gly Gln Val Ala Glu					
	660	665	670				
cag gag gag gct	gct gcc ctg cgg	caa gcc cta acc	ctc tcc ctg ctg	2185			
Gln Glu Glu Ala	Ala Ala Leu Arg	Gln Ala Leu Thr Leu Ser Leu Leu					
	675	680	685				
gag cag ccc ccg	ttg gag gca gaa	gaa ccc cca gat	ggg ggg act gat	2233			
Glu Gln Pro Pro	Leu Glu Ala Glu	Glu Pro Pro Asp Gly Gly Thr Asp					
	690	695	700				
ggc aag gcc cag	ctg gtg gtg cac	tcg gcc ttt gag	cag gat gtg gag	2281			
Gly Lys Ala Gln	Leu Val Val His Ser Ala Phe	Glu Gln Asp Val Glu					
	705	710	715				
gag ctg gac cgg	gcg ctc agg gct	gcc ttg gag gtc	cac gtc cag gag	2329			
Glu Leu Asp Arg	Ala Leu Arg Ala	Ala Leu Glu Val His Val Gln Glu					
	725	730	735				
gag acg gtg ggg	ccc tgg cgc cgc	aca ctg cct gca	gag ctg cgt gct	2377			
Glu Thr Val Gly	Pro Trp Arg Arg Thr Leu Pro	Ala Glu Leu Arg Ala					
	740	745	750				
cgc ctg gag cgg	tgc cat ggt gtg	agt gtt gcc ctg	cgt ggt gac tgc	2425			
Arg Leu Glu Arg	Cys His Gly Val Ser Val Ala Leu	Arg Gly Asp Cys					
	755	760	765				
acc atc ctc cgt	ggc ttc ggg gcc	cac cct gcc cgt	gct gcc cgc cac	2473			
Thr Ile Leu Arg	Gly Phe Gly Ala His Pro Ala	Arg Ala Ala Arg His					
	770	775	780				
ttg gtg gca ctt	ctg gct ggc ccc	tgg gat cag agt	ttg gcc ttt ccc	2521			
Leu Val Ala Leu	Leu Ala Gly Pro Trp Asp Gln Ser Leu	Ala Phe Pro					
	785	790	800				
ttg gca gct tca	ggc cct acc ttg	gcg ggg cag acg	ctg aag ggg ccc	2569			
Leu Ala Ala Ser	Gly Pro Thr Leu Ala Gly Gln Thr Leu Lys Gly Pro						
	805	810	815				
tgg aac aac ctg	gag cgt ctg gca	gag aac acc ggg	gag ttc cag gag	2617			
Trp Asn Asn Leu	Glu Arg Leu Ala Glu Asn Thr Gly Glu Phe Gln Glu						
	820	825	830				
gtg gtg cgg gcc	ttc tac gac acc	ctg gac gct gcc	cgc agc agc atc	2665			
Val Val Arg Ala	Phe Tyr Asp Thr Leu Asp Ala Ala Arg Ser Ser Ile						
	835	840	845				
cgc gtc gtt cgt	gtg gag cgc gtg	tcg cac ccg ctg	ctg cag cag cag	2713			
Arg Val Val Arg	Val Glu Arg Val Ser His Pro Leu Leu Gln Gln Gln						
	850	855	860				
tat gag ctg tac	cgg gag cgc ctg	ctg cag cga tgc	gag cgg cgc ccg	2761			
Tyr Glu Leu Tyr	Arg Glu Arg Leu Leu Gln Arg Cys Glu Arg Arg Pro						
	865	870	875				
gtg gag cag gtg	ctg tac cac ggc	acg acg gca ccg	gca gtg cct gac	2809			

Val Glu Gln Val Leu Tyr His Gly Thr Thr Ala Pro Ala Val Pro Asp  
885 890 895

atc tgc gcc cac ggc ttc aac cgc agc ttc tgc ggc cgc aac gcc acg 2857  
Ile Cys Ala His Gly Phe Asn Arg Ser Phe Cys Gly Arg Asn Ala Thr  
900 905 910

gtc tac ggg aag ggc gtg tat ttc gcc agg cgc gcc tcc ctg tcg gtg 2905  
Val Tyr Gly Lys Gly Val Tyr Phe Ala Arg Arg Ala Ser Leu Ser Val  
915 920 925

cag gac cgc tac tcg ccc ccc aac gcc gat ggc cat aag gcg gtg ttc 2953  
Gln Asp Arg Tyr Ser Pro Pro Asn Ala Asp Gly His Lys Ala Val Phe  
930 935 940

gtg gca cgg gtg ctg act ggc gac tac ggg cag ggc cgc cgc ggt ctg 3001  
Val Ala Arg Val Leu Thr Gly Asp Tyr Gly Gln Gly Arg Arg Gly Leu  
945 950 955 960

cgg gcg ccc cct ctg cgg ggt cct ggc cac gtg ctc ctg cgc tac gac 3049  
Arg Ala Pro Pro Leu Arg Gly Pro Gly His Val Leu Leu Arg Tyr Asp  
965 970 975

agc gcc gtg gac tgc atc tgc cag ccc agc atc ttc gtc atc ttc cac 3097  
Ser Ala Val Asp Cys Ile Cys Gln Pro Ser Ile Phe Val Ile Phe His  
980 985 990

gac acc cag gcg ctg ccc acc cac ctc atc acc tgc gag cac gtg ccc 3145  
Asp Thr Gln Ala Leu Pro Thr His Leu Ile Thr Cys Glu His Val Pro  
995 1000 1005

cgc gct tcc ccc gac gac ccc tct ggg ctc ccg ggc cgc tcc cca gac 3193  
Arg Ala Ser Pro Asp Asp Pro Ser Gly Leu Pro Gly Arg Ser Pro Asp  
1010 1015 1020

act taaccgaagg ggccaccctc tggcctcctg cttcccaggc tcccagctcc 3246  
Thr  
1025

gcacaggctg atgctccccg cccccaactg tggccgctg agctgtcccc ggggacgccc 3306

ctgcctccct ctgcgggctc cagaaggcgg tgtgggggat ggcggtcagc agcggccgag 3366

aggggctggg ctaggtccca gcttgggccc accccaccac caggggtcag cagagcccag 3426

gaggcgacac tgcccgcgccc cgcctccag acctcgccc agtcggctct gttgtttgaa 3486

taaactgtaa cgtgaaccca ggcggaagg acccgggaaa aaaaaaaaaa aaaaa 3541

&lt;210&gt; 2

&lt;211&gt; 1025

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 2

Met Val Ala Met Ala Glu Ala Glu Ala Gly Val Ala Val Glu Val Arg  
1 5 10 15

Gly Leu Pro Pro Ala Val Pro Asp Glu Leu Leu Thr Leu Tyr Phe Glu  
20 25 30

Asn Arg Arg Arg Ser Gly Gly Gly Pro Val Leu Ser Trp Gln Arg Leu  
                   35                                  40                                  45  
 Gly Cys Gly Gly Val Leu Thr Phe Arg Glu Pro Ala Asp Ala Glu Arg  
           50                                  55                                  60  
 Val Leu Ala Gln Ala Asp His Glu Leu His Gly Ala Gln Leu Ser Leu  
   65                                  70                                  75                                  80  
 Arg Pro Ala Pro Pro Arg Ala Pro Ala Arg Leu Leu Leu Gln Gly Leu  
                                   85                                  90                                  95  
 Pro Pro Gly Thr Thr Pro Gln Arg Leu Glu Gln His Val Gln Ala Leu  
                                  100                                 105                                 110  
 Leu Arg Ala Ser Gly Leu Pro Val Gln Pro Cys Cys Ala Leu Ala Ser  
           115                                 120                                 125  
 Pro Arg Pro Asp Arg Ala Leu Val Gln Leu Pro Lys Pro Leu Ser Glu  
   130                                 135                                 140  
 Ala Asp Val Arg Val Leu Glu Glu Gln Ala Gln Asn Leu Gly Leu Glu  
  145                                 150                                 155                                 160  
 Gly Thr Leu Val Ser Leu Ala Arg Val Pro Gln Ala Arg Ala Val Arg  
                                  165                                 170                                 175  
 Val Val Gly Asp Gly Ala Ser Val Asp Leu Leu Leu Leu Glu Leu Tyr  
                                  180                                 185                                 190  
 Leu Glu Asn Glu Arg Arg Ser Gly Gly Gly Pro Leu Glu Asp Leu Gln  
   195                                 200                                 205  
 Arg Leu Pro Gly Pro Leu Gly Thr Val Ala Ser Phe Gln Gln Trp Gln  
   210                                 215                                 220  
 Val Ala Glu Arg Val Leu Gln Gln Glu His Arg Leu Gln Gly Ser Glu  
  225                                 230                                 235                                 240  
 Leu Ser Leu Val Pro His Tyr Asp Val Leu Glu Pro Glu Glu Leu Ala  
                                  245                                 250                                 255  
 Glu Asn Thr Ser Gly Gly Asp His Pro Ser Thr Gln Gly Pro Arg Ala  
                                  260                                 265                                 270  
 Thr Lys His Ala Leu Leu Arg Thr Gly Gly Leu Val Thr Ala Leu Gln  
           275                                 280                                 285  
 Gly Ala Gly Thr Val Thr Met Gly Ser Gly Glu Glu Pro Gly Gln Ser  
   290                                 295                                 300  
 Gly Ala Ser Leu Arg Thr Gly Pro Met Val Gln Gly Arg Gly Ile Met  
  305                                 310                                 315                                 320  
 Thr Thr Gly Ser Gly Gln Glu Pro Gly Gln Ser Gly Thr Ser Leu Arg  
                                  325                                 330                                 335  
 Thr Gly Pro Met Gly Ser Leu Gly Gln Ala Glu Gln Val Ser Ser Met  
                                  340                                 345                                 350  
 Pro Met Gly Ser Leu Glu His Glu Gly Leu Val Ser Leu Arg Pro Val  
   355                                 360                                 365

Gly Leu Gln Glu Gln Glu Gly Pro Met Ser Leu Gly Pro Val Gly Ser  
 370 375 380  
 Ala Gly Pro Val Glu Thr Ser Lys Gly Leu Leu Gly Gln Glu Gly Leu  
 385 390 395 400  
 Val Glu Ile Ala Met Asp Ser Pro Glu Gln Glu Gly Leu Val Gly Pro  
 405 410 415  
 Met Glu Ile Thr Met Gly Ser Leu Glu Lys Ala Gly Pro Val Ser Pro  
 420 425 430  
 Gly Cys Val Lys Leu Ala Gly Gln Glu Gly Leu Val Glu Met Val Leu  
 435 440 445  
 Leu Met Glu Pro Gly Ala Met Arg Phe Leu Gln Leu Tyr His Glu Asp  
 450 455 460  
 Leu Leu Ala Gly Leu Gly Asp Val Ala Leu Leu Pro Leu Glu Gly Pro  
 465 470 475 480  
 Asp Met Thr Gly Phe Arg Leu Cys Gly Ala Gln Ala Ser Cys Gln Ala  
 485 490 495  
 Ala Glu Glu Phe Leu Arg Ser Leu Leu Gly Ser Ile Ser Cys His Val  
 500 505 510  
 Leu Cys Leu Glu His Pro Gly Ser Ala Arg Phe Leu Leu Gly Pro Glu  
 515 520 525  
 Gly Gln His Leu Leu Gln Gly Leu Glu Ala Gln Phe Gln Cys Val Phe  
 530 535 540  
 Gly Thr Glu Arg Leu Ala Thr Ala Thr Leu Asp Thr Gly Leu Glu Glu  
 545 550 555 560  
 Val Asp Pro Thr Glu Ala Leu Pro Val Leu Pro Gly Asn Ala His Thr  
 565 570 575  
 Leu Trp Thr Pro Asp Ser Thr Gly Gly Asp Gln Glu Asp Val Ser Leu  
 580 585 590  
 Glu Glu Val Arg Glu Leu Leu Ala Thr Leu Glu Gly Leu Asp Leu Asp  
 595 600 605  
 Gly Glu Asp Trp Leu Pro Arg Glu Leu Glu Glu Glu Gly Pro Gln Glu  
 610 615 620  
 Gln Pro Glu Glu Glu Val Thr Pro Gly His Glu Glu Glu Glu Pro Val  
 625 630 635 640  
 Ala Pro Ser Thr Val Ala Pro Arg Trp Leu Glu Glu Glu Ala Ala Leu  
 645 650 655  
 Gln Leu Ala Leu His Arg Ser Leu Glu Pro Gln Gly Gln Val Ala Glu  
 660 665 670  
 Gln Glu Glu Ala Ala Ala Leu Arg Gln Ala Leu Thr Leu Ser Leu Leu  
 675 680 685  
 Glu Gln Pro Pro Leu Glu Ala Glu Glu Pro Pro Asp Gly Gly Thr Asp  
 690 695 700

Gly Lys Ala Gln Leu Val Val His Ser Ala Phe Glu Gln Asp Val Glu  
 705 710 715 720  
 Glu Leu Asp Arg Ala Leu Arg Ala Ala Leu Glu Val His Val Gln Glu  
 725 730 735  
 Glu Thr Val Gly Pro Trp Arg Arg Thr Leu Pro Ala Glu Leu Arg Ala  
 740 745 750  
 Arg Leu Glu Arg Cys His Gly Val Ser Val Ala Leu Arg Gly Asp Cys  
 755 760 765  
 Thr Ile Leu Arg Gly Phe Gly Ala His Pro Ala Arg Ala Ala Arg His  
 770 775 780  
 Leu Val Ala Leu Leu Ala Gly Pro Trp Asp Gln Ser Leu Ala Phe Pro  
 785 790 795 800  
 Leu Ala Ala Ser Gly Pro Thr Leu Ala Gly Gln Thr Leu Lys Gly Pro  
 805 810 815  
 Trp Asn Asn Leu Glu Arg Leu Ala Glu Asn Thr Gly Glu Phe Gln Glu  
 820 825 830  
 Val Val Arg Ala Phe Tyr Asp Thr Leu Asp Ala Ala Arg Ser Ser Ile  
 835 840 845  
 Arg Val Val Arg Val Glu Arg Val Ser His Pro Leu Leu Gln Gln Gln  
 850 855 860  
 Tyr Glu Leu Tyr Arg Glu Arg Leu Leu Gln Arg Cys Glu Arg Arg Pro  
 865 870 875 880  
 Val Glu Gln Val Leu Tyr His Gly Thr Thr Ala Pro Ala Val Pro Asp  
 885 890 895  
 Ile Cys Ala His Gly Phe Asn Arg Ser Phe Cys Gly Arg Asn Ala Thr  
 900 905 910  
 Val Tyr Gly Lys Gly Val Tyr Phe Ala Arg Arg Ala Ser Leu Ser Val  
 915 920 925  
 Gln Asp Arg Tyr Ser Pro Pro Asn Ala Asp Gly His Lys Ala Val Phe  
 930 935 940  
 Val Ala Arg Val Leu Thr Gly Asp Tyr Gly Gln Gly Arg Arg Gly Leu  
 945 950 955 960  
 Arg Ala Pro Pro Leu Arg Gly Pro Gly His Val Leu Leu Arg Tyr Asp  
 965 970 975  
 Ser Ala Val Asp Cys Ile Cys Gln Pro Ser Ile Phe Val Ile Phe His  
 980 985 990  
 Asp Thr Gln Ala Leu Pro Thr His Leu Ile Thr Cys Glu His Val Pro  
 995 1000 1005  
 Arg Ala Ser Pro Asp Asp Pro Ser Gly Leu Pro Gly Arg Ser Pro Asp  
 1010 1015 1020  
 Thr  
 1025

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
21 March 2002 (21.03.2002)

PCT

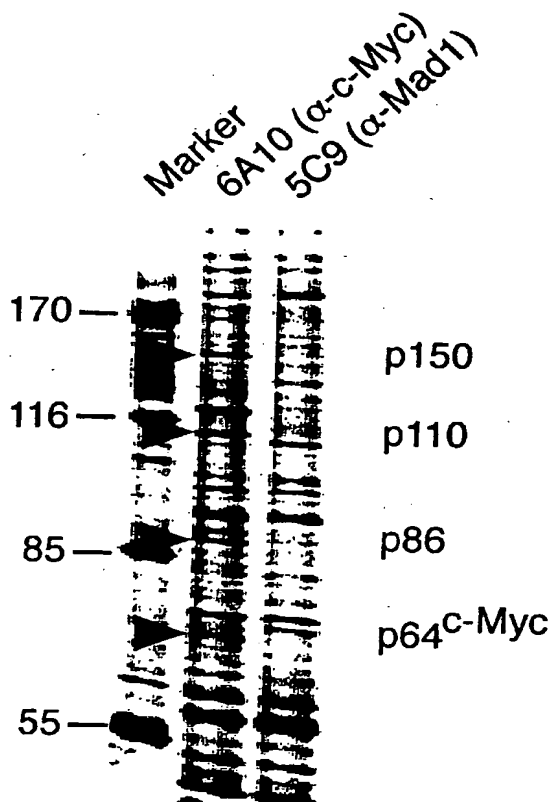
(10) International Publication Number  
**WO 02/022792 A3**

- (51) International Patent Classification<sup>7</sup>: C12N 9/10, A61K 38/45 (74) Agent: VOSSIUS & PARTNER; Siebertstrasse 4, 81675 Munich (DE).
- (21) International Application Number: PCT/EP01/10494 (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (22) International Filing Date: 11 September 2001 (11.09.2001)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: 00119849.8 12 September 2000 (12.09.2000) EP (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- 01113577.9 13 June 2001 (13.06.2001) EP
- (71) Applicants and  
(72) Inventors: LÜSCHER, Bernhard [CH/DE]; Schwanenring 21 A, 30627 Hannover (DE). AUSTEN, Matthias [DE/US]; Wacholderweg, 24, 37079 Göttingen (DE).

[Continued on next page]

(54) Title: NUCLEIC ACID MOLECULES ENCODING A POLY(ADP-RIBOSE) POLYMERASE

(57) Abstract: Disclosed are nucleic acid molecules encoding a protein having poly(ADP-ribose) polymerase (PARP) activity as well as the encoded protein. Furthermore, the invention describes expression vectors, host cells, antibodies, pharmaceutical compositions and methods for treating disorders associated with aberrant regulation of cellular behaviour. Finally, screening methods are described for compounds that act as agonist or antagonist of the protein having PARP activity.



WO 02/022792 A3

WO 02/022792 A3



**Published:**

— with international search report

**(88) Date of publication of the international search report:**

25 July 2002

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 01/10494

A. CLASSIFICATION OF SUBJECT MATTER  
 IPC 7 C12N9/10 A61K38/45

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

MEDLINE, EPO-Internal, WPI Data, PAJ, CHEM ABS Data, EMBASE, SCISEARCH, SEQUENCE SEARCH, BIOSIS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1 033 401 A (GENSET SA) 6 September 2000 (2000-09-06) the whole document	1-5, 7, 9, 14
X	-& DATABASE GENSEQ PROTEIN 'Online! DERWENT; AC: AAG04073, 6 September 2000 (2000-09-06) DUMAS ET AL.: "Human; 5' EST; expressed sequence tag; secreted protein; cDNA isolation; gene therapy; chromosome mapping" XP002195076 the whole document	1-5, 7, 9, 14



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*&\* document member of the same patent family

Date of the actual completion of the international search

4 April 2002

Date of mailing of the international search report

22/04/2002

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
 NL - 2280 HV Rijswijk  
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
 Fax: (+31-70) 340-3016

Authorized officer

Steffen, P

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 01/10494

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online! EBI; AC: AL040630, 12 March 1999 (1999-03-12) KOEHRER ET AL: "EST; expressed sequence tag" XP002195066 cited in the application the whole document	1-5,9,14
X	--- DATABASE EMBL 'Online! EBI; AC: AI002047, 11 June 1998 (1998-06-11) NCI-CGAP: "EST" XP002195067 cited in the application the whole document	1-5,9,14
X	--- DATABASE EMBL 'Online! EBI; AC: AL040631, 12 March 1999 (1999-03-12) KOEHRER ET AL.: "EST; expressed sequence tag" XP002195068 the whole document	1-5,9,14
P,X	--- DATABASE GENESEQ NUCLEOTIDE 'Online! Derwent; AC: AAH15559, 26 June 2001 (2001-06-26) OTA ET AL.: "Human cDNA sequence SEQ ID NO: 13847" XP002195069 the whole document	1-5,9,14
P,X	--- & DATABASE GENSEQ PROTEIN 'Online! Derwent; AAB93889, 26 June 2001 (2001-06-26) OTA ET AL.: "Human protein sequence SEQ ID NO: 13848" XP002195070 the whole document	7
P,X	& EP 1 074 617 A (HELIX RESEARCH INSTITUTE) 7 February 2001 (2001-02-07) abstract	1-5,7,9, 14
P,X	--- DATABASE EMBL 'Online! EBI; AC: AK027370, 15 May 2001 (2001-05-15) ISOGAI ET AL.: "f1s (full insert sequence)" XP002195071 the whole document	1-5,9,14
	--- -/--	

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 01/10494

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>D'AMOURS DAMIEN ET AL: "Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions." BIOCHEMICAL JOURNAL, vol. 342, no. 2, pages 249-268, XP002195062 ISSN: 0264-6021 cited in the application the whole document</p> <p>---</p>	
A	<p>KICKHOEFER VALERIE A ET AL: "The 193-kD vault protein, VPARP, is a novel poly(ADP-ribose) polymerase." JOURNAL OF CELL BIOLOGY, vol. 146, no. 5, pages 917-928, XP002195063 ISSN: 0021-9525 cited in the application the whole document</p> <p>---</p>	
A	<p>AME JEAN-CHRISTOPHE ET AL: "PARP-2, a novel mammalian DNA damage-dependent poly(ADP-ribose) polymerase." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 274, no. 25, 18 June 1999 (1999-06-18), pages 17860-17868, XP002195064 ISSN: 0021-9258 cited in the application the whole document</p> <p>-----</p>	

INTERNATIONAL SEARCH REPORT

International application No.

EP01/10494

ADDITIONAL MATTER PCT/ISA/210

Continuation of box I.2

Claims nos. 11-13

Present claims nos 11-13 relate to a product which is respectively characterized by a desirable characteristic or property, i.e. the product is an unspecified antagonist of the protein of the present application.

The patent claims thus include all products etc. which exhibit this characteristic or property. However, in the application the description only provides support for a limited number of such products according to the terms of PCT Article 5. In the present case, the patent claims lack the appropriate support and the patent application lacks the required disclosure to such an extent that a meaningful search encompassing the entire scope of protection sought seems impossible. Moreover, the patent claims lack clarity as required in PCT Article 6, an attempt being made therein to to define the product by means of the respectively desired result. The lack of clarity is such that a meaningful search of the entire scope of protection sought is rendered impossible. As a result, no meaningful search could be carried out for claims nos. 11-13.

The applicant's attention is drawn to the fact that claims, or parts of claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e)PCT). EPO policy, when acting as an International Preliminary Examining Authority, is normally not to carry out a preliminary examination on matter which has not been searched. This is the case, irrespective of whether or not the claims are amended following receipt of the search report (Article 19 PCT) or during any Chapter II procedure whereby the applicant provides new claims.

# INTERNATIONAL SEARCH REPORT

**International Application No**

PCT/EP 01/10494

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 1033401 A	06-09-2000	EP 1033401 A2 JP 2001269182 A	06-09-2000 02-10-2001

# INTERNATIONALER RECHERCHENBERICHT

Internationales Aktenzeichen

PCT/EP 01/10494

A. KLASSIFIZIERUNG DES ANMELDUNGSGEGENSTANDES  
IPK 7 C12N9/10 A61K38/45

Nach der Internationalen Patentklassifikation (IPK) oder nach der nationalen Klassifikation und der IPK

## B. RECHERCHIERTE GEBIETE

Recherchierte Mindestprüfstoff (Klassifikationssystem und Klassifikationssymbole)  
IPK 7 C12N

Recherchierte aber nicht zum Mindestprüfstoff gehörende Veröffentlichungen, soweit diese unter die recherchierten Gebiete fallen

Während der internationalen Recherche konsultierte elektronische Datenbank (Name der Datenbank und evtl. verwendete Suchbegriffe)

MEDLINE, EPO-Internal, WPI Data, PAJ, CHEM ABS Data, EMBASE, SCISEARCH, SEQUENCE SEARCH, BIOSIS

## C. ALS WESENTLICH ANGESEHENE UNTERLAGEN

Kategorie*	Bezeichnung der Veröffentlichung, soweit erforderlich unter Angabe der in Betracht kommenden Teile	Beitr. Anspruch Nr.
X	EP 1 033 401 A (GENSET SA) 6. September 2000 (2000-09-06) das ganze Dokument	1-5,7,9, 14
X	-& DATABASE GENSEQ PROTEIN 'Online! DERWENT; AC: AAG04073, 6. September 2000 (2000-09-06) DUMAS ET AL.: "Human; 5' EST; expressed sequence tag; secreted protein; cDNA isolation; gene therapy; chromosome mapping" XP002195076 das ganze Dokument	1-5,7,9, 14

☒ Weitere Veröffentlichungen sind der Fortsetzung von Feld C zu entnehmen

☒ Siehe Anhang Patentfamilie

\* Besondere Kategorien von angegebenen Veröffentlichungen :

\*A\* Veröffentlichung, die den allgemeinen Stand der Technik definiert, aber nicht als besonders bedeutsam anzusehen ist

\*E\* älteres Dokument, das jedoch erst am oder nach dem internationalen Anmeldedatum veröffentlicht worden ist

\*L\* Veröffentlichung, die geeignet ist, einen Prioritätsanspruch zweifelhaft erscheinen zu lassen, oder durch die das Veröffentlichungsdatum einer anderen im Recherchenbericht genannten Veröffentlichung belegt werden soll oder die aus einem anderen besonderen Grund angegeben ist (wie ausgeführt)

\*O\* Veröffentlichung, die sich auf eine mündliche Offenbarung, eine Benutzung, eine Ausstellung oder andere Maßnahmen bezieht

\*P\* Veröffentlichung, die vor dem internationalen Anmeldedatum, aber nach dem beanspruchten Prioritätsdatum veröffentlicht worden ist

\*T\* Spätere Veröffentlichung, die nach dem internationalen Anmeldedatum oder dem Prioritätsdatum veröffentlicht worden ist und mit der Anmeldung nicht kollidiert, sondern nur zum Verständnis des der Erfindung zugrundeliegenden Prinzips oder der ihr zugrundeliegenden Theorie angegeben ist

\*X\* Veröffentlichung von besonderer Bedeutung; die beanspruchte Erfindung kann allein aufgrund dieser Veröffentlichung nicht als neu oder auf erfinderischer Tätigkeit beruhend betrachtet werden

\*Y\* Veröffentlichung von besonderer Bedeutung; die beanspruchte Erfindung kann nicht als auf erfinderischer Tätigkeit beruhend betrachtet werden, wenn die Veröffentlichung mit einer oder mehreren anderen Veröffentlichungen dieser Kategorie in Verbindung gebracht wird und diese Verbindung für einen Fachmann naheliegend ist

\*Z\* Veröffentlichung, die Mitglied derselben Patentfamilie ist

Datum des Abschlusses der internationalen Recherche

4. April 2002

Absendedatum des internationalen Recherchenberichts

22/04/2002

Name und Postanschrift der Internationalen Recherchenbehörde  
Europäisches Patentamt, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Bevollmächtigter Bediensteter

Steffen, P

C.(Fortsetzung) ALS WESENTLICH ANGESEHENE UNTERLAGEN		
Kategorie*	Bezeichnung der Veröffentlichung, soweit erforderlich unter Angabe der in Betracht kommenden Teile	Betr. Anspruch Nr.
X	<p>DATABASE EMBL 'Online!  EBI;  AC: AL040630, 12. März 1999 (1999-03-12)  KOEHRER ET AL: "EST; expressed sequence tag"  XP002195066  in der Anmeldung erwähnt  das ganze Dokument</p>	1-5,9,14
X	<p>DATABASE EMBL 'Online!  EBI;  AC: AI002047, 11. Juni 1998 (1998-06-11)  NCI-CGAP: "EST"  XP002195067  in der Anmeldung erwähnt  das ganze Dokument</p>	1-5,9,14
X	<p>DATABASE EMBL 'Online!  EBI;  AC: AL040631, 12. März 1999 (1999-03-12)  KOEHRER ET AL.: "EST; expressed sequence tag"  XP002195068  das ganze Dokument</p>	1-5,9,14
P,X	<p>DATABASE GENESEQ NUCLEOTIDE 'Online!  Derwent;  AC: AAH15559, 26. Juni 2001 (2001-06-26)  OTA ET AL.: "Human cDNA sequence SEQ ID NO: 13847"  XP002195069  das ganze Dokument</p>	1-5,9,14
P,X	<p>-&amp; DATABASE GENESEQ PROTEIN 'Online!  Derwent;  AAB93889, 26. Juni 2001 (2001-06-26)  OTA ET AL.: "Human protein sequence SEQ ID NO: 13848"  XP002195070  das ganze Dokument</p>	7
P,X	<p>&amp; EP 1 074 617 A (HELIX RESEARCH INSTITUTE) 7.-Februar 2001 (2001-02-07)  Zusammenfassung</p>	1-5,7,9,14
P,X	<p>DATABASE EMBL 'Online!  EBI;  AC: AK027370, 15. Mai 2001 (2001-05-15)  ISOGAI ET AL.: "fis (full insert sequence)"  XP002195071  das ganze Dokument</p>	1-5,9,14

-/--

# INTERNATIONALER RECHERCHENBERICHT

### Internationales Aktenzeichen

PCT/EP 01/10494

**C.(Fortsetzung) ALS WESENTLICH ANGESEHENE UNTERLAGEN**

Kategorie*	Bezeichnung der Veröffentlichung, soweit erforderlich unter Angabe der in Betracht kommenden Teile	Betr. Anspruch Nr.
A	<p>D'AMOURS DAMIEN ET AL:            "Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions."            BIOCHEMICAL JOURNAL,            Bd. 342, Nr. 2, Seiten 249-268,            XP002195062            ISSN: 0264-6021            in der Anmeldung erwähnt            das ganze Dokument</p> <p>----</p>	
A	<p>KICKHOEFER VALERIE A ET AL: "The 193-kD vault protein, VPARP, is a novel poly(ADP-ribose) polymerase."            JOURNAL OF CELL BIOLOGY,            Bd. 146, Nr. 5, Seiten 917-928,            XP002195063            ISSN: 0021-9525            in der Anmeldung erwähnt            das ganze Dokument</p> <p>----</p>	
A	<p>AME JEAN-CHRISTOPHE ET AL: "PARP-2, a novel mammalian DNA damage-dependent poly(ADP-ribose) polymerase."            JOURNAL OF BIOLOGICAL CHEMISTRY,            Bd. 274, Nr. 25,            18. Juni 1999 (1999-06-18), Seiten            17860-17868, XP002195064            ISSN: 0021-9258            in der Anmeldung erwähnt            das ganze Dokument</p> <p>-----</p>	

WEITERE ANGABEN

PCT/ISA/ 210

Fortsetzung von Feld I.2

Ansprüche Nr.: 11-13

Die geltenden Patentansprüche 11-13 beziehen sich auf ein Produkt, jeweils charakterisiert durch eine erstrebenswerte Eigenheit oder Eigenschaft, nämlich dass das Produkt ein unspezifizierter Antagonist des Proteins der vorliegenden Anmeldung ist.

Die Patentansprüche umfassen daher alle Produkte etc., die diese Eigenheit oder Eigenschaft aufweisen, wohingegen die Patentanmeldung Stütze durch die Beschreibung im Sinne von Art. 5 PCT nur für eine begrenzte Zahl solcher Produkte etc. liefert. Im vorliegenden Fall fehlen den Patentansprüchen die entsprechende Stütze bzw. der Patentanmeldung die nötige Offenbarung in einem solchen Maße, daß eine sinnvolle Recherche über den gesamten erstrebten Schutzbereich unmöglich erscheint. Desungeachtet fehlt den Patentansprüchen auch die in Art. 6 PCT geforderte Klarheit, nachdem in ihnen versucht wird, das Produkt über das jeweils erstrebte Ergebnis zu definieren. Auch dieser Mangel an Klarheit ist dergestalt, daß er eine sinnvolle Recherche über den gesamten erstrebten Schutzbereich unmöglich macht. Daher konnte keine sinnvolle Recherche für die Ansprüche 11-13 durchgeführt werden.

Der Anmelder wird darauf hingewiesen, daß Patentansprüche, oder Teile von Patentansprüchen, auf Erfindungen, für die kein internationaler Recherchenbericht erstellt wurde, normalerweise nicht Gegenstand einer internationalen vorläufigen Prüfung sein können (Regel 66.1(e) PCT). In seiner Eigenschaft als mit der internationalen vorläufigen Prüfung beauftragte Behörde wird das EPA also in der Regel keine vorläufige Prüfung für Gegenstände durchführen, zu denen keine Recherche vorliegt. Dies gilt auch für den Fall, daß die Patentansprüche nach Erhalt des internationalen Recherchenberichtes geändert wurden (Art. 19 PCT), oder für den Fall, daß der Anmelder im Zuge des Verfahrens gemäß Kapitel II PCT neue Patentansprüche vorlegt.

# INTERNATIONALER RECHERCHENBERICHT

Internationales Aktenzeichen

PCT/EP 01/10494

Im Recherchenbericht angeführtes Patentdokument	Datum der Veröffentlichung	Mitglied(er) der Patentfamilie	Datum der Veröffentlichung
EP 1033401 A	06-09-2000	EP 1033401 A2	06-09-2000
		JP 2001269182 A	02-10-2001

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☒ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**

**THIS PAGE BLANK (USPTO)**